THE ROLE OF ANGIOTENSIN II IN THE REGULATION OF RENAL FUNCTION

By K.D. MITCHELL, B.Sc.

A thesis presented for the Degree
of Doctor of Philosophy
at the University of Edinburgh

Department of Physiology,
University Medical School,
Teviot Place,
Edinburgh.

December, 1985.

EX BIBL. UNIV. EDINBURGEN.

UNIVERSITY OF EDINBURGH

ABSTRACT OF THESIS (Regulation 7.9)

Name of Candidate KENNETH D. MITCHELL

Address .....

Degree Ph.D. *Date* DECEMBER 1985

Title of Thesis THE ROLE OF ANGIOTENSIN II IN THE REGULATION OF RENAL FUNCTION

Previous studies have provided evidence that the renin-angiotensin system exerts numerous physiological actions. In this thesis, attention is focussed on the direct actions of exogenous and endogenous angiotensin II (AII) in the regulation of renal function. A review of the literature pertaining to the biochemistry of the renin-angiotensin system, the actions of angiotensin II and angiotensin III (AIII) on renal function, the driving forces for fluid absorption by the proximal tubule and some of the factors which influence proximal tubular fluid absorption is presented.

The experiments described in this thesis were designed to evaluate the effects of angiotensin II on renal function in the anaesthetised rat prepared for micropuncture.

Systemic infusion of a pressor dose (100 $ng.min^{-1}$) of AII resulted in a marked increase in both urine flow and urinary sodium excretion. These changes were associated with a significant reduction in proximal tubular fluid reabsorption. However, when the renal perfusion pressure was prevented from increasing, the same dose of AII resulted in a significant reduction in both urine flow and urinary sodium excretion. These changes occurred in the absence of a significant alteration in the rate of proximal tubular fluid reabsorption. These results indicate that the natriuretic response to AII is dependent upon the increase in renal perfusion pressure.

Infusion of sub-pressor doses (5 and 10 $ng.min^{-1}$) of AII resulted in a subtle antidiuresis and antinatriuresis when the effects of the peptide were compared to the effects of infusion of saline alone. In addition, infusion of AII at a rate of 10 $ng.min^{-1}$ significantly reduced glomerulur filtration rate. However, infusion of these sub-pressor doses of AII did not alter proximal tubular fluid reabsorption significantly.

Further experiments were carried out in which rats were acutely volume expanded (infusion 0.9% NaCl I.V. at a rate of 150 $\mu l.min^{-1}$) and/or chronically sodium loaded (replacing drinking water with saline solution) in an attempt to suppress endogenous angiotensin production and thereby demonstrate a pronounced antinatriuretic response to infused hormone. Infusion of sub-pressor doses (0.1, 1 and 5 $ng.min^{-1}$) of AII in chronically sodium loaded rats did not significantly alter urine flow, urinary sodium excretion, glomerular filtration rate or proximal tubular fluid reabsorption.

In acutely volume expanded chronically sodium loaded rats, infusion of AII at rates of 5 and 10 $ng.min^{-1}$ resulted in significant reductions in urine flow and urinary sodium excretion. These changes occurred in the absence of significant alterations in glomerular filtration rate or proximal tubular fluid reabsorption. Under the same conditions, infusion of the converting enzyme inhibitor, captopril (SQ14,225), resulted in a significant increase in urinary sodium excretion and a reduction in the percentage of filtered sodium reabsorbed. Infusion of AII (10 $ng.min^{-1}$) during continuing converting enzyme blockade resulted in a significant antidiuresis and antinatriuresis. These excretory responses were associated with a significant decrease in glomerular filtration rate and a restoration of fractional sodium reabsorption to pre-captopril levels.

These results are consistent with the concept that the anaesthetised rat produces amounts of angiotensin that are almost optimal for maximal stimulation of proximal reabsorption and sodium retention and that it is necessary to suppress endogenous angiotensin production in order to demonstrate a pronounced antinatriuretic effect of infused hormone. In addition, the results provide no evidence in support of the concept that the mechanism whereby systemic AII reduces urine flow and sodium excretion involves a direct stimulatory effect of the peptide on proximal tubular fluid reabsorption.

I, Kenneth D. Mitchell, hereby declare that this thesis, entitled "The Role of Angiotensin II in the Regulation of Renal Function", submitted for the Degree of Doctor of Philosophy at the University of Edinburgh, has been composed by myself and is the result of work done entirely by myself.

KENNETH D. MITCHELL

December, 1985

CONTENTS

SECTION 2 - METHODS

SECTION 3 - PRESSURE DEPENDENCE OF ANGIOTENSIN-INDUCED NATRIURESIS IN RATS

ACKNOWLEDGEMENTS

I would like to express my thanks to Dr. Peter J. Harris for his supervision and encouragement during my postgraduate research training; to Dr. John A. Russell for many helpful discussions; and to Dr. L. Gabriel Navar (Dept. of Physiology and Biophysics, Nephrology Research and Training Center, University of Alabama at Birmingham, Birmingham, Alabama, U.S.A.) for his help with the manuscript.

The secretarial assistance of Mrs. Joyce Kanute is gratefully acknowledged.

I would like to express my special gratitude to my mother for her support and encouragement throughout my education.

The experimental work described in this thesis was carried out during the tenure of a Faculty of Medicine Postgraduate fellowship.

Part of this work has been published in abstract form (J. Physiol. 332, 93-94P, 1982; Renal Physiol. 7 (4),253, 1984). The abstracts are included as an appendix.

INTRODUCTION

The components of the renin-angiotensin system are summarised in Fig. 1. Renin is an acid protease which cleaves renin substrate (angiotensinogen) to produce the decapeptide angiotensin I (AI), which is then converted to the octapeptide angiotensin II (AII) by the dipeptidase, angiotensin converting enzyme. An aspartate aminopeptidase, angiotensinase A, cleaves the NH_2-terminal aspartate residue from AII to form the heptapeptide angiotensin III (AIII).

Although it is generally accepted that the major physiological actions of the renin-angiotensin system are mediated by AII, the heptapeptide AIII does exert a wide range of activity in various tissues.

The renin-angiotensin system is thought to be involved in the regulation of arterial blood pressure and extracellular fluid volume. Extracellular fluid volume depletion, caused by a variety of circumstances, leads to the activation of numerous mechanisms involved with the conservation of water and electrolytes and with body fluid and arterial pressure homeostasis (De Wardener, 1978; Navar, 1984). One of the more powerful of these is the renin-angiotensin system, which serves as a highly sensitive index of the state of sodium balance (Davis and Freeman, 1976; Aguilera and Catt, 1978). AII is a major stimulus for the biosynthesis and secretion of the sodium-retaining steroid aldosterone (Laragh et al., 1960; Biron et al., 1961; Page and Bumpus, 1974; Aguilera and Catt, 1978; Mendelsohn and Kachel, 1980), and much of it's sodium conserving influence is mediated by the renal effects of aldosterone.

Aldosterone influences sodium and potassium transport in the distal tubule and collecting duct segments by altering the activity of the Na^+/K^+-ATPase located predominantly on the basolateral side of tubular cells and also by facilitating Na^+/K^+ exchange processes on the luminal membranes (Ludens and Fanestil, 1976; Edelman, 1979). However, the

action of aldosterone is relatively slow and requires a latent period of 45 to 120 minutes before onset (Barger et al., 1958; Ganong and Mulrow, 1958; Ludens, and Fanestil, 1976).

It is generally accepted that sodium depletion or volume contraction results in increases in circulating renin, AII and aldosterone levels and an increase in the sensitivity of the aldosterone secretory response to AII. Conversely, sodium loading or extracellular fluid volume expansion results in decreases in circulating renin, AII and aldosterone levels and a decrease in the sensitivity of the steroidogenic response to AII. Directional changes in the number of adrenal AII receptors parallel the changes in adrenal responsiveness to AII (Douglas and Catt, 1976; Aguilera and Catt, 1978). Several studies have documented that circulating AII is a major regulator of adrenal AII receptor number during changes in sodium balance and that changes in AII binding are a major determinant of altered responsiveness to AII (Douglas and Catt, 1976; Aguilera and Catt, 1978; Hauger et al, 1978; Douglas and Brown, 1982). Thus, as a major physiological regulator of aldosterone biosynthesis and secretion, AII can be considered an important hormone influencing the basal long-term sodium transport activity of the distal nephron during varying states of sodium balance.

However, it is well recognised that the actions of AII are not restricted to stimulation of aldosterone secretion. AII acts directly on cardiac and vascular smooth muscle to stimulate contraction, and increases circulating catecholamines by an action on autonomic ganglia, sympathetic nerve endings and the adrenal medulla (Farr and Grupp, 1971; Peach, 1971; Bonnardeaux and Regoli, 1974; Regoli et al., 1974). AII acts on the central nervous system to increase sympathetic nervous system activity and thus arterial blood pressure (Bickerton and Buckley, 1961; Severs et al., 1966; Reid et al., 1982; Hartle and Brody, 1984). Other actions of the peptide on the CNS include stimulation of thirst, salt appetite, arginine vasopressin release and inhibition of renin and

aldosterone secretion (Malvin, 1971; Keil et al., 1975; Chiaraviglio, 1976; Fitzsimons et al., 1978; Brooks and Malvin, 1980; Reid et al., 1982).

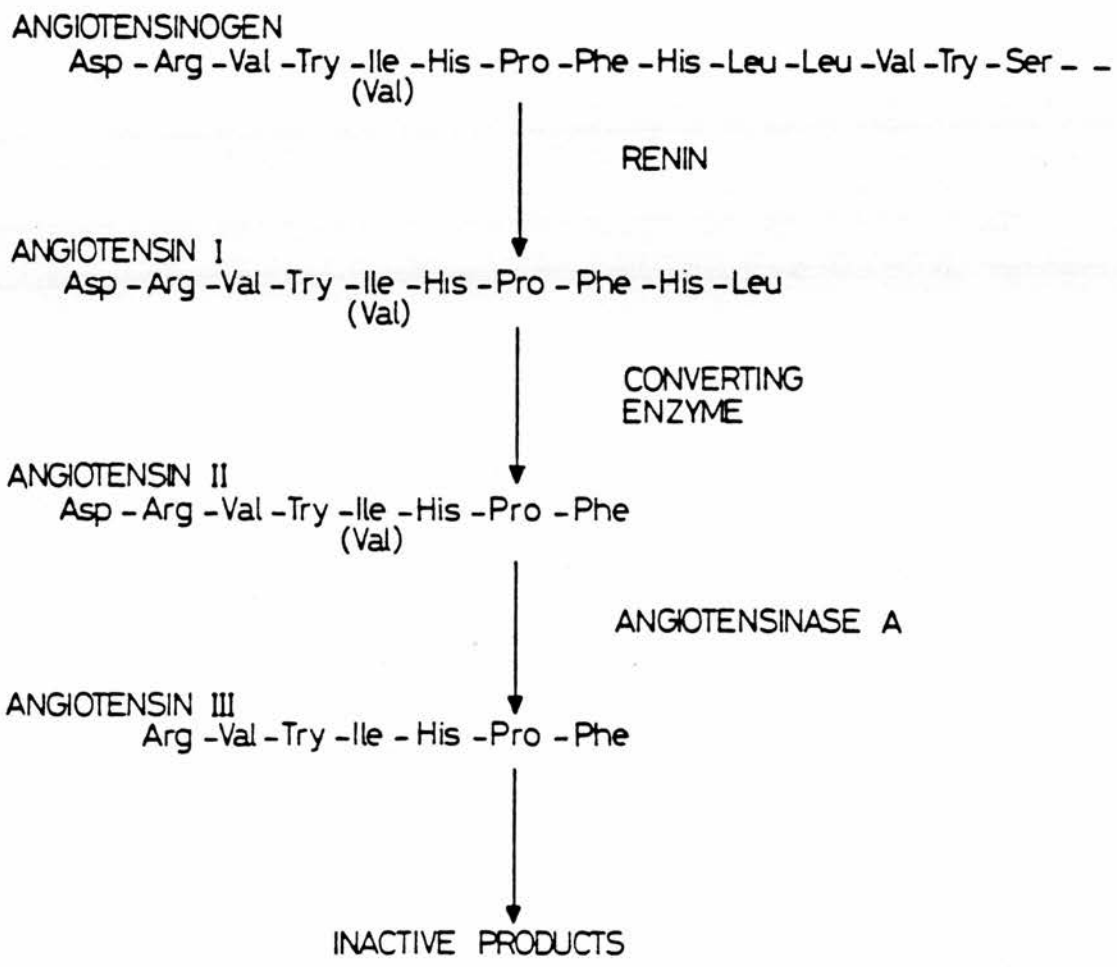
In addition, AII has been reported to alter both electrogenic and electroneutral salt transport by various epithelial tissues (McAfee and Locke, 1967; Davies et al., 1970; Bolton et al., 1975; Healy et al., 1976; Harris and Young, 1977; Levens et al., 1981b; Schuster et al., 1984). Furthermore, it has been demonstrated that the peptide exerts direct effects on the kidney to alter the renal excretion of sodium and water (Barraclough, 1965; Barraclough et al., 1967; Navar and Langford, 1974; Levens et al., 1981a).

Although the direct effects of AII on renal function have been studied extensively (Navar and Langford, 1974; Levens et al., 1981a) there is still controversy as to the principal intrarenal site of action of AII and the contribution of the renin-angiotensin system to the regulation of renal function.

This thesis is concerned with the role of AII in the regulation of renal function. In Section I, attention is focussed on the different components of the renin-angiotensin system and the effects of angiotensins II and III on renal function. Because part of the work of this thesis is concerned with the effects of systemic infusions of AII on proximal tubular fluid reabsorption, there is also a brief review of the driving forces for fluid absorption by the proximal tubule and some of the factors involved in the control of proximal tubular reabsorption.

The experimental work described in Sections 3-6 was designed to evaluate the effects of AII on renal function in the anaesthetised rat prepared for micropuncture, and to assess the possible mechanism (s) whereby AII alters renal function. The results from these experiments are discussed in relation to the concept that AII exerts major actions in the regulation of renal function.

FIG I THE RENIN-ANGIOTENSIN SYSTEM



SECTION 1

LITERATURE REVIEW

RENIN SUBSTRATE (ANGIOTENSINOGEN)

DISTRIBUTION

Renin substrate or angiotensinogen is a glycoprotein normally present in the blood and is part of the α_2-globulin fraction of plasma (Plentl et al., 1943). Renin substrate was first assumed to be synthesised by the liver by Page et al., (1941) and Leloir et al., (1942). This was later demonstrated in the isolated perfused liver (Nasjletti and Masson, 1971), in rat liver slices (Freeman and Rostorfer, 1972) and in isolated hepatocytes (Weigand et al., 1977). Ganten et al., (1972) reported renin substrate in dog brain, but showed that none of the components of the renin-angiotensin system could cross the blood brain barrier and concluded that there was an intrinsic angiotensin producing system in the brain. Renin substrate has also been detected in the renal lymph of rats (Horky et al., 1971) and in specific subcellular granules in rat kidney cortex (Morris and Johnston, 1976). More recently, using immunocytochemistry, Richoux et al., (1983) demonstrated that renin substrate was present in rat kidney as granular formations in the cells of proximal convoluted tubules, although no substrate was detected in the juxtaglomerular apparatus. However, it is not known whether the presence of substrate in the kidney is due to local synthesis or to uptake from the plasma.

PURIFICATION AND STRUCTURE

Renin substrate was first purified by Skeggs et al., (1963) from hog plasma. Using a purification process which included acidification to pH 2.5, five different forms of substrate were isolated, all of which were glycoproteins with a molecular weight of approximately 58,000. The different forms had similar amino acid compositions but varied in their glucosamine, neutral hexose and sialic acid content (Skeggs et al., 1963).

Recently both rat and human renin substrate were purified (Printz and Printz, 1977; Eggena et al., 1978; Hilgenfeldt and Hackenthal, 1979; Tewksbury et al., 1981). Both were found to be glycoproteins, with molecular weights varying from 52-60,000 depending on the technique used, and with different isoelectric points ranging from 4.3 to 5.0. Two major forms of renin substrate have been purified from rat plasma (Bouhnik et al., 1981; Hilgenfeldt and Hackenthal, 1982; Voigt et al., 1982). Similar heterogeneity was observed in the substrate in both the CSF and plasma of man and rat, suggesting that the observed differences were not due to isolation artifacts. Physiological variations in renin substrate such as the increases induced by bilateral nephrectomy or oestrogen administration were found not to alter its physicochemical characteristics (Menard et al., 1974a) although forms of substrate with molecular weights exceeding 55-77,000 were found during pregnancy (Gordon et al., 1976) and oestrogen treatment (Tewksbury and Dart, 1981).

The renin substrate molecule from horse plasma has been degraded with trypsin to yield the NH_2-terminal tetradecapeptide which is hydrolysed by renin at the Leu^{10}-Leu^{11} bond to yield AI (Skeggs et al., 1957) (Fig.1). The structure of the tetradecapeptide has been determined and the molecule synthesised. The tetradecapeptide isolated possessed full substrate activity (Skeggs et al., 1958). The structure of the NH_2-terminal sequence has also been determined in both rat and human substrate (Bouhnik et al., 1981; Tewksbury et al., 1981). The structure of the last ten amino acids corresponds to that of horse AI although human substrate has a renin cleavage site different from hog and rat substrate.

The structural requirements of renin for its substrate have been studied by comparison of the activity of renin on various peptide analogues representing portions of the tetradecapeptide (Skeggs et al.,

1968; Marshall, 1976). Several synthetic peptides have proved effective inhibitors of renin, both in vivo and in vitro (Burton et al., 1980; Szelke et al., 1982).

PHYSIOLOGICAL ROLE OF RENIN SUBSTRATE

An alteration in renin substrate level may lead to changes in renal function and arterial blood pressure as a result of altered AI and AII production. When renin substrate level increases, the negative feedback which normally exists between AII and renin secretion should rapidly reduce renin secretion (De Champlain et al., 1966) so that the AII production level is kept constant while the level of plasma renin substrate is higher and that of plasma renin lower. However, in reality, this does not happen and the physiological effects of variations in renin substrate on renal function and blood pressure have been well documented. Indeed, elevated plasma AII levels and reduced renal blood flow were reported in non-hypertensive women receiving oestroprogestational contraceptives, ie, in women with elevated plasma substrate levels (Menard et al., 1974b; Hollenberg et al., 1976). Also, renin substrate was found to cause renal vasoconstriction in the rabbit kidney and the isolated perfused rat kidney (Krahe et al., 1970; Regoli, 1972). In addition, the role of renin substrate in the maintenance of arterial blood pressure in the rat was demonstrated by the reduction in blood pressure induced by the injection of renin substrate antibodies (Gardes et al., 1982).

VARIATIONS IN PLASMA CONCENTRATION

Plasma renin substrate concentration decreases after adrenalectomy, hepatectomy and hypophysectomy (Nasjletti and Masson, 1971; Hasegawa et al., 1973; Reid et al., 1973) and increases after bilateral nephrectomy (Leloir et al., 1940; Carretero and Gross, 1967), during pregnancy

(Gould et al., 1966; Helmer and Judson, 1967), in malignant hypertension (Gould and Green, 1971; Rosset et al., 1973), in severe hypoxia (Gould and Goodman, 1970) and after administration of ACTH, adrenal cortical hormones, oestrogens and AII (Helmer and Griffith, 1952; Menard et al., 1970; Krakoff and Eisenfeld, 1977; Reid, 1977). The mechanism controlling plasma substrate level is not known, although it has been proposed that it involves regulation of hepatic synthesis and not the rate of utilisation by circulating renin (Nasjletti and Masson, 1971; 1972; 1973; Bing, 1972).

RENIN

Tigerstedt and Bergman (1898) first used the name renin to indicate a saline-extractable pressor substance obtained from kidney homogenates. In 1934, Goldblatt et al., demonstrated that hypertension could be induced in experimental animals by the partial ligation of a renal artery. They also showed that the manoeuvre resulted in the appearance in the renal venous blood of a directly pressor substance on injection into other animals. However, the mechanism of renin's action remained unclear until Page and Helmer (1940) demonstrated that it was probably an enzyme which acted upon another substance to release an active principle which they called angiotonin. Braun-Menendez et al., (1940) working independently made a similar observation, but named the active principle hypertensin. The active substance was later called angiotensin, a synthesis of the terms angiotonin and hypertensin.

Renin catalyses the formation of a decapeptide, AI (Skeggs et al., 1954) which is the first in a series of peptides generated by a cascade of enzymes working in concert, ie, the renin-angiotensin system cascade (Fig.1).

DISTRIBUTION

Renin (renin isoenzymes) has been found in many different tissues, although it is predominantly located in the kidney.

It is generally accepted that renin is synthesised and stored by specialised cells, the juxtaglomerular cells, of the renal afferent arteriole in intimate contact with the distal tubule (Goormaghtigh, 1939; Edelman and Hartroft, 1961; Robertson et al., 1965; Johnston et al., 1973; Silverman and Barajas 1974; Taugner et al., 1982a; 1982b). The juxtaglomerular cells are thought to be modified smooth muscle cells because they contain bundles of fibrils and attachment bodies similar to

those of smooth muscle cells (Latta, 1973). As the distal tubule approaches the vascular pole of the glomerulus it comes into direct contact with the juxtaglomerular cells and the arterioles. The distal tubule cells are modified and those in apposition to the glomerulus are columnar in shape and are densely packed. This appearance gives rise to the term macula densa cells. Collectively the juxtaglomerular cells, the afferent glomerular arteriole and the macula densa segment of the distal tubule are termed the juxtaglomerular apparatus (Hatt, 1967; Barajas, 1970; Rouiller and Orci, 1971; Latta, 1973).

Using micropuncture and microdissection techniques, it has been shown that renin is released from the cells of the afferent arteriole (juxtaglomerular cells) and that renin activity is absent from other cells of the juxtaglomerular apparatus (Faarup, 1968; 1971; Ogino et al., 1967). Similar findings were reported by Hartroft et al., (1964) using immunological techniques combined with fluorescence microscopy. Cook (1967) further localised renin to the granules within the juxtaglomerular cells themselves by removing the granules from these cells with a micro-pipette. On incubation of the granules with renin substrate angiotensin was produced, thus demonstrating the presence of renin activity in the granules. The granularity within the juxtaglomerular cells has been used as an index of their synthetic and secretory activity (Davis and Freeman, 1976).

Renin is released from the granules into the afferent arteriolar lumen (Horky et al., 1971) and probably also into the interstitial space (Horky et al., 1973; Morgan and Davis, 1975; Morgan and Gillies, 1977). The secretion of renin into the renal interstitium would allow enough time for the local generation of AI and AII and would be consistent with an action of the renin-angiotensin system within the kidney. The findings that the renal tissue content of renin decreases from the superficial to deep layers of the cortex and that renin release is

higher in the superficial than in the deep cortical and medullary nephrons (Peart, 1959a; Brown et al., 1966; Jones et al., 1979) suggests that AII may not be formed homogeneously throughout the kidney.

Significant amounts of renin activity have been found in the uterus and placenta (Gross et al., 1964; Ferris et al., 1967; Anderson et al., 1968; Carretero et al., 1971). The renin from these two tissues is similar to renal renin (Anderson et al., 1968; Ryan and Johnson, 1969). Both uterine and placental renin increase during pregnancy (Ferris et al., 1967; Hodari et al., 1969; Chesley, 1974). Although plasma renin activity varies greatly during pregnancy in mammals, most human studies show that plasma renin activity is increased during normal pregnancy (Skinner et al., 1972; Weir et al., 1975). Plasma renin is also increased at ovulation and during the luteal phase of the menstrual cycle. However, it is not known how much of the increase in plasma renin during the menstrual cycle and pregnancy is of uterine or uteroplacental origin.

Renin-like activity was first discovered in the rat and dog brain by Ganten et al., (1971) and independently by Fischer-Ferraro et al., (1971). In 1976, Day and Reid raised the question of whether brain renin was in fact Cathepsin D (an acid protease normally present in lysosomes) and thus raised doubts about the physiological significance of the reported brain renin. However, using affinity ion exchange chromatography, Ganten and Speck, (1978), Hirose et al., (1978), Osman et al., (1979), Inagami et al., (1980), independently showed that Cathepsin D could be separated from brain renin and that the latter had the ability to form AI at a neutral pH. Thus, it is now generally accepted that there is a brain renin separate from the acid protease Cathepsin D.

Since renin does not penetrate the blood-brain barrier and renin activity persists in the brain after nephrectomy, it is likely that the

enzyme is synthesised within the brain itself (Fischer-Ferraro et al., 1971; Ganten et al., 1972). The concept of a renin generating system within the brain is supported by several studies which demonstrated the presence of renin (and other components of the renin-angiotensin system) in neuroblastoma cells lines (Fishman et al., 1981; Inagami et al., 1982).

Werle et al., (1957) reported extremely high concentrations of renin-like activity in the submaxillary gland of the mouse. This enzyme has been purified and reported to have a molecular weight of 42,000 (Werle et al., 1968; Cohen et al., 1972). Recently Misono et al., (1982) determined the complete amino acid sequence of mouse submaxillary gland renin. They found that, of the amino acids, 43% were identical to those in porcine pepsin. Although the physiological role of mouse submaxillary gland renin is not known, it shares many characteristic properties with mouse renal renin including immunological reactivity (Michelakis et al., 1974).

Brown et al., (1964a) failed to find sufficient renin activity in the blood of hypertensive subjects to account for the elevation of blood pressure. This led to the search for the enzyme in the blood vessel wall. Indeed, renin activity has been found in the large arteries and veins of the hog and dog (Dengler, 1956; Gould et al., 1964). This renin-like activity is similar to renal renin. Ganten et al., (1970) found renin activity in the plasma of 24-hour nephrectomised dogs and that this activity increased after haemorrhage. At the same time renin activity in portal vein and splanchnic blood of such animals increased at a faster rate than in arterial blood. From these findings they concluded that the splanchnic bed has the capacity to synthesise and secrete renin into the general circulation.

PURIFICATION AND PROPERTIES OF RENIN

Various techniques have been employed to isolate renin from kidneys. Studies in which acid conditions were used have resulted in multiple proteins with renin-like activity (Skeggs et al., 1967). Using gel filtration and ultracentrifugation techniques, Levine et al., (1970) and Rubin (1972) showed that the molecular weight of the predominant species of hog renal renin was 40,000. A major protein component isolated by affinity chromatography of hog kidney was demonstrated to have a molecular weight of 40,000 (Murakami and Inagami, 1975). In addition, Waldhausl et al., (1970) reported a molecular weight of 42,000 for human renal renin. Recently, using improved gel filtration and affinity chromatographic techniques, renal renins purified from hog, dog, rat and human kidneys were found to have molecular weights of approximately 40,000 (Corvol et al., 1977; Inagami and Murakami, 1977; Dzau et al., 1979; Galen et al., 1979; Yokosawa et al., 1980; Atlas et al., 1981). These enzymes were found to have a broad pH optimum in the pH 5.6 - 7.0 range when tested with homologous substrate, although both higher and lower pH optima were observed with heterologous or synthetic peptide substrates. Human kidney renin was found to have a somewhat narrower pH optimum with homologous substrate at about pH 6 (Yokosawa et al., 1980).

The molecular weight of human plasma renin as measured by gel filtration has been reported to be approximately 50,000 (Takii et al., 1980; Atlas et al. 1981), which is somewhat higher than the molecular weight of 40,000 reported for purified human renal renin (Inagami and Murakami, 1977; Corvol et al., 1977; Dzau et al., 1979; Galen et al., 1979; Yokosawa et al., 1980; Atlas et al., 1981).

INACTIVE RENIN

An inactive form of renin in human amniotic fluid was first reported by Lumbers (1971). She showed that amniotic fluid dialysed to a pH of 3.3 and then back to a pH of 7.4 had a higher renin concentration than amniotic fluid dialysed to a pH above 4.0, and suggested that this effect was due to activation of an inactive form of renin by acid. Since then, there have been many reports of high-molecular weight forms of renin with low enzymatic activity in amniotic fluid, plasma and renal tissue. This new aspect of the renin problem has acquired a degree of complexity which is reflected in the nomenclature since the high-molecular weight forms of renin have been given different names by different research groups, eg, "big renin" (Day and Luetscher, 1974), "inactive renin" (Leckie and McConnell, 1975; Skinner et al., 1975) and "prorenin" (Sealey et al., 1976). I do not intend to review this subject and readers are referred to the following comprehensive review articles (Inagami and Murakami, 1980; Sealey et al., 1980;1983; Leckie, 1981).

CONVERTING ENZYME

Renin cleaves the α_2-globulin angiotensinogen to produce the decapeptide AI that is subsequently converted to the highly active octapeptide AII by a peptidyl dipeptide hydrolase also know as angiotensin-converting enzyme. Yang et al., (1970) demonstrated that the dipeptide hydrolase, kininase II, which inactivates the potent vasodepressor peptides bradykinin and kallidin is the same enzyme as angiotensin-converting enzyme.

OCCURRENCE OF THE ENZYME

Converting enzyme was first isolated from horse plasma by Skeggs et al., (1954). The role of this enzyme was not appreciated until Helmer (1955) using in vitro aortic strips, showed that AI was inactive until exposed to plasma, whereas AII was active without prior exposure to plasma. Following the report by Ng and Vane (1967) that the conversion of AI to AII by the plasma enzyme was too slow to account for the very rapid action of AI in vivo, it was established that the lungs contained a high level of converting enzyme activity (Bakhle, 1968; Ng and Vane, 1968).

It has been found that in the lung, the surface of the vascular endothelial cells is rich in enzyme (Bakhle, 1968; Ryan et al., 1971; 1975; Smith et al., 1973; Ryan and Ryan, 1980), and that converting enzyme is probably located on the pinocytotic vesicles of the endothelial cells. These results together with the finding that there was substantial conversion of AI during a single passage through the lung (Bakhle et al., 1969; Aiken and Vane, 1970; Ryan et al., 1971; Biron and Campeau, 1971; Igic et al., 1972), suggested that the lung was the most important site for conversion of AI.

However, converting enzyme has also been found in many different tissues in several species (Roth et al., 1969; Cushman and Cheung, 1971 a; Horky et al., 1971; Poth et al., 1975). For example, high enzyme activity has been found in rat testicles (Cushman and Cheung, 1971 a), rat lymph (Horky et al., 1971), human and rat brain (Yang and Neff, 1972; Poth et al., 1975) and rabbit liver, adrenal cortex, pancreas and spleen (Caldwell et al., 1976).

Converting enzyme has also been reported in the kidney. Using fluorescent immunohistochemistry, it was demonstrated that converting enzyme was present in the endothelial cells of glomerular and inter-glomerular capillaries and on the epithelial cells of the proximal tubules of rabbit kidneys. Converting enzyme activity has been localised in the brush border membrane from proximal tubules in the kidney (Ward et al., 1977; Caldwell et al., 1976; Ryan et al., 1976; Takada et al., 1982). More recently, using improved immunocytochemical methods, converting enzyme was found in specific endothelial cells in the rat kidney (Taugner et al., 1982a). Enzyme activity was found in the endothelial cells of all kidney arteries, the afferent and efferent arterioles, some glomerular capillaries and the first branches of the peritubular capillaries, although no activity was observed in renal veins or renin producing granulated cells (Taugner et al., 1982a). The presence of converting enzyme activity in the kidney has also been demonstrated by the conversion of AI to AII during passage through the kidney, with estimates of the magnitude of this conversion ranging from 1 to 20% (Ng and Vane, 1968; Franklin et al., 1970; Di Salvo et al., 1971; Hofbauer et al., 1976; Britton, 1981). Recently Rosivall et al., (1983) reported that mean conversion of AI to AII during a single passage through the kidney was about 22% and that this did not change under conditions in which renin secretion and AI generation rate increased. All these results support the concept that AII is formed not

only systemically, but also intrarenally from blood borne and/or intrarenally generated AI.

PURIFICATION AND PROPERTIES OF THE ENZYME

Converting enzyme has been purified from plasma, lung and kidney from several species including human, guinea pig, hog, rabbit, calf and rat (Lee et al., 1971; Eliseeva et al., 1971; Cushman and Cheung, 1972; Dorer et al., 1972; Fitz and Overturf, 1972; Igic et al., 1972; Stevens et al., 1972; Nakajima et al., 1973; Oshima et al., 1974; 1976; Das and Soffer, 1975). The molecular weights found for the enzyme isolated from rabbit serum, human and hog plasma, human, hog, guinea pig, calf and rat lungs range between 140,000 and 480,000 (Lee et al., 1971; Dorer et al., 1972; Fitz and Overturf, 1972; Stevens et al., 1972; Lanzillo and Fanburg, 1974; 1976; Oshima et al., 1974; Nishimura et al., 1976; 1978; Das et al., 1977). The enzyme from the rabbit lung was found to be a glycoprotein of molecular weight 129,000 consisting of a single polypeptide chain, a polysaccharide residue accounting for 26% of the dry weight, and one zinc atom per molecule (Soffer et al., 1974; Das and Soffer, 1975). The enzymes isolated from rabbit serum, guinea pig and human plasma, guinea pig and human lung and hog kidney were also found to be glycoproteins and to contain zinc (Oshima et al., 1974; 1976; Lanzillo and Fanburg, 1976; 1977; Das et al., 1977; Nishimura et al., 1978).

Converting enzyme is a nonspecific dipeptidylcarboxypeptidase which will cleave a wide variety of peptides including AI, bradykinin and leu-&-met enkephalin by removing dipeptide fragments from the carboxyl-terminal (Igic et al., 1972; Erdos, 1975; Skeggs et al., 1976).

There are several general requirements for catalytic activity. The carboxyl terminal residue must be an L - amino acid with a free carboxyl function. An acidic amino acid at the carboxyl-terminus reduces the

susceptibility of the substrate to hydrolysis (Orekhovich, 1968). The enzyme does not cleave a peptide that has a penultimate proline residue, and hence converting enzyme does not cleave AII (Ackerly and Peach, 1975).

Although the enzyme hydrolyses molecules as small as tripeptides (Erdos, 1975), only (des-Asp^1) AI among the carboxyl-terminal homologues of AI, is a physiological substrate for converting enzyme (Chiu et al., 1976; Tsai et al., 1975).

The rates of hydrolysis of various peptide substrates by converting enzyme are markedly affected by the presence of monovalent anions such as chloride (Cushman and Cheung, 1971b; Erdos, 1975; Cushman et al., 1978). For example, the hydrolysis of AI stops entirely in chloride free medium whereas that of bradykinin continues at about 35-50% of the optimum rate (Igic et al., 1973; Dorer et al., 1974). Chloride has been described as an allosteric modifier of converting enzyme and chloride ions lower the Km of the enzyme for substrate (Cheung and Cushman, 1973; Oshima et al., 1974).

INHIBITORS

Converting enzyme is a metalloenzyme inhibited by metal complexing agents such as EDTA, EGTA and dimercaptopropanol and by cations such as Ni^{2+},Cd^{2+},Hg^{2+},Pb^{2+} and Cu^{2+}, that may serve to replace the zinc ions of the enzyme to form a catalytically ineffective metalloenzyme (Huggins et al., 1970; Cushman and Cheung, 1971b; Dorer et al., 1976). Inhibition by chelators can be reversed by the addition of Co^{2+},Ca^{2+} or Mn^{2+}. Converting enzyme is also inhibited to varying degrees by peptides that may bind to the enzyme in the same manner as substrate or the polypeptide or dipeptide products of the enzyme's action (Erdos, 1975; Massey and Fessler, 1976; Tsai and Peach, 1977; Cushman et al., 1978).

For example, products of the hydrolysis of AI and (des - Asp^1) AI, such as the dipeptide His - Leu; the heptapeptide fragment from the hydrolysis of (des - Asp^1) AI (AIII) and the hexa- and heptapeptides of AII inhibit converting enzyme (Tsai et al., 1975; Chiu et al., 1976; Tsai and Peach, 1977; Cushman et al., 1978).

It was discovered that the venom of the Brazilian 'arrowhead' viper BOTHROPS jararaca contained an active peptide fraction that potentiated the in vitro action of bradykinin (Ferreira, 1965; Ferreira and Rocha e Silva, 1965). The active peptide fraction which was called 'Bradykinin - potentiating factor' or BPF was later shown to augment in vivo the cardiovascular, CNS and antinociceptive actions of bradykinin (Ferreira, 1965; Ferreira and Rocha e Silva, 1965; Graeff et al., 1965; Amorim et al., 1967; Ribeiro et al., 1971). Similar BPF fractions were isolated from other BOTHROPS species and from the Japanese viper AGKISTRODON halys blomhoffi (Ferreira, 1965; 1966). In 1968, Bakhle reported that BPF inhibited angiotensin converting enzyme activity of dog lung homogenate. This was followed by the finding that BPF inhibited conversion of AI to AII in the perfused guinea pig lung and in rats in vivo (Bakhle et al., 1969; Ng and Vane, 1970). A number of the component peptides from both BOTHROPS and AGKISTRODON venoms have been isolated, sequenced and synthesised (Greene et al., 1969; Ferreira et al., 1970; Kato and Suzuki, 1971; Ondetti et al., 1971; Stewart et al., 1971; Cheung and Cushman, 1973).

Of those peptides, the first to be synthesised was the pentapeptide BPF_{5a} (Stewart et al., 1971), which was found to be a good inhibitor in vitro and also a substrate of the enzyme (Aiken and Vane, 1970; Cushman and Cheung, 1971b; Yang et al., 1971; Cheung and Cushman, 1973). The nonapeptide SQ 20,881 (BPF_{9a}, teprotide) and the undecapeptide 'Potentiator C', also inhibit the enzyme but also contain a carboxyl-terminal Pro-Pro dipeptide that is not cleaved by converting enzyme. Thus, they

inhibit the hydrolysis of bradykinin and AI as competitive reversible inhibitors without being substrates of the enzyme themselves (Igic et al., 1972; Cheung and Cushman, 1973). Thus, peptide inhibitors can either be a substrate for converting enzyme (as with BPF_{5a}) or not (as with SQ 20,881).

The nonapeptide SQ 20,881 and other peptide inhibitors have been used in humans and animals to explore the importance of the functions of the renin-angiotensin system under a variety of conditions.

The recently discovered orally active inhibitor of converting enzyme, captopril (D-3-mercapto-2-methylpropanoyl-L-proline; SQ 14,225), has been used in the diagnosis and therapy of several forms of human hypertension (Ondetti et al., 1977; Gavras et al., 1978; Rubin et al., 1978; Brunner et al., 1979). However, several serious side-effects including maculopapillary rash, glomerulonephritis, proteinuria and neutropenia were associated with the drug (Heel et al., 1980; Alexander and Meyer, 1981). These side-effects were attributed to the sulphydryl moiety of the drug (Heel et al., 1980). This has led to the development of orally effective non-sulphydryl converting enzyme inhibitors. Recently the diacids MK 421 and SCH 31846 have been described. Both belong to the class of carboxyalkyldipeptide non-sulphydryl converting enzyme inhibitors (Patchett et al., 1980; Gross et al., 1981; Sweet et al., 1981; Baum et al., 1983; Sybertz et al., 1983).

ANGIOTENSINASES

Angiotensinases are peptides or proteolytic enzymes that catalyse the hydrolysis of peptide bonds in AII. Three general types of angiotensinase activity have been described: aminopeptidase, acting on the NH_2-terminal end of the molecule (Glenner et al., 1962; Khairallah et al., 1963; Khairallah and Page, 1967; Nagatsu et al., 1970); endopeptidase, acting on the tyrosine^4 - isoleucine^5 bond to form two tetrapeptides (Khairallah et al., 1963; Regoli et al., 1963; Matsunaga and Masson, 1970) and carboxypeptidase, acting on the COOH-terminal end of the AII molecule (Johnson and Ryan, 1968; Yang et al., 1968; Matsunaga and Masson, 1970).

AMINOPEPTIDASES

Glenner et al., (1962) first identified aminopeptidase activity in a microsomal preparation from rat kidney homogenates. Two AII degrading enzymes were then found in plasma (Khairallah et al., 1963; Nagatsu et al., 1970), and called angiotensinase A_1, and angiotensinase A_2. Angiotensinase A_1 was found to be an aminopeptidase selective for an NH_2- terminal asparaginyl residue, was inhibited by EDTA, had a pH optimum of 7.4 and was stable at 60°C for 30 minutes (Khairallah and Page, 1967). Angiotensinase A_2 however, was found to be an aminopeptidase selective for an NH_2-terminal aspartyl residue. This enzyme was also inhibited by EDTA, had a pH optimum of 6.8 and was heat labile (Khairallah and Page, 1967).

Khairallah et al., (1963) identified an aminopeptidase in red blood cells that seemed to correspond to plasma angiotensinase. Both were found to be inhibited by epsilon aminocaproic acid, Hg^{2+} and Cd^{2+} (Itskovitz and Miller, 1967). The significant type of angiotensinase activity reported in red blood cells, aminopeptidase, was reported to be

confined to the inside of the red blood cell membrane (Moore et al., 1977). However, the functional role of this enzyme is still not known since it is unclear whether or not the red cell enzyme has access to circulating AII.

Aminopeptidase activity has been reported in the proximal tubules and juxtaglomerular apparatus (JG) of the kidney. This activity, which is present in the microsomal fraction, has a neutral pH optimum and requires Ca^{2+} (Hess, 1965; Itskovitz and Miller, 1966; Granger et al., 1969; Pullman et al., 1975). However, since this enzyme was found not to hydrolyse (des - Asp^{1}) AI, while the isolated perfused kidney and kidney in vivo does degrade this nonapeptide, it is unlikely that this enzyme is physiologically the most important aminopeptidase (Leary and Ledingham, 1970b; Cheung and Cushman, 1971; Oparil et al., 1971; Oparil and Bailie, 1973; Misumi et al., 1983).

Aminopeptidase activity has been reported in brain homogenates, intestine, spleen, adrenal, pancreas, lung and vascular tissue (Itskovitz and Miller, 1967; Abrash et al., 1971; Ledingham and Leary, 1974; Palaic and Farrugia, 1980). It is difficult to assign any physiological importance to many of these aminopeptidases. For example, although lung homogenates rapidly degrade AII, very little AII, if any, is degraded in the intact lung (Itskovitz and Miller, 1967; Leary and Ledingham, 1969). However, the vascular response to angiotensin can be enhanced by using angiotensinase inhibitors, the membrane bound angiotensin in vascular tissue shows identical binding properties for AII as angiotensin receptors, and the aminopeptidase on the outside of adrenal cells catalyses the conversion of AII to AIII. These findings suggest that the tissue bound aminopeptidases in vascular and adrenal cells may be important in angiotensin receptor operation and regulation, and for AII induced aldosterone secretion (Blair-West et al., 1971a;

Campbell et al., 1974; Peach and Chiu, 1974; Goodfriend and Peach, 1975; Le Morvan et al., 1977; Moore et al., 1977; Farruggia et al., 1979).

ENDOPEPTIDASES (ANGIOTENSINASE B)

In 1963, Regoli et al., isolated from plasma kidney homogenates an endopeptidase that was active at at acidic pH and degraded AII into two tetrapeptides. Khairallah and Page (1967) identified an enzyme from plasma which hydrolysed analogues of AII that were resistant to aminopeptidase hydrolysis. This enzyme was inhibited by diisopropylphosphofluoridate (DFP) and it was suggested that this was an acid endopeptidase and was labelled "angiotensinase B".

McDonald et al., (1974) isolated an enzyme in the lysosomal fraction of rat liver which hydrolysed AII. This enzyme had a pH optium of 5.0 and had a greater hydrolytic activity on peptides with penultimate basic residues. This enzyme was found to be a dipeptidylaminopeptidase (Cathepsin C).

CARBOXYPEPTIDASES (ANGIOTENSINASE C)

Yang et al., (1968) first obtained a carboxypeptidase capable of hydrolysing AII from urine. This enzyme (soluble) of renal origin was stable at 60°C, was inhibited by DFP and released phenylalanine from AII. This enzyme was originally named "angiotensinase C", and has now been characterised as a prolylcarboxypeptidase specific for the hydrolysis of COOH-terminal amino acids with a penultimate prolyl residue and splits the -Pro^{7} - Phe^{8} bond of AII (Kakimoto et al., 1973). This enzyme has been found in the lysosomes of the kidney and spleen. This enzyme was purified from kidney cortex and was found to have a molecular weight of 210,000 (Kakimoto et al., 1973). It is doubtful whether this heat stable enzyme accounts for much of the total angiotensinase activity in the kidney, since Johnson and Ryan (1968) showed that

over 90% of the total renal activity was inactivated by heating at 56°C for 30 minutes. Johnson and Ryan (1968) also found evidence of carboxypeptidase activity in aqueous extracts of rabbit liver. This enzyme accounted for 30% of hepatic angiotensinase activity. It is similar to prolylcarboxypeptidase in that it is active at acid pH, is heat stable and is inhibited by DFP.

Goldstein et al., (1972) isolated a carboxypeptidase from brain tissue of rat and dog. However, this enzyme differs from the prolylcarboxypeptidase in that it was found to degrade AI and AII, whereas the prolylcarboxypeptidases do not cleave AI.

HALF-LIFE OF ANGIOTENSIN II

The half-life of AII in vivo is approximately one minute in the human and in the order of 20 - 30 seconds in the rat, and most disappears during a single passage through the vascular beds of various regions and organs in the systemic circulation (Hodge et al., 1967; Biron et al., 1968; Doyle et al., 1968; Leary and Ledingham, 1969; Ledingham and Leary, 1974; Ryan, 1974; Al-Merani et al., 1978; Brooks et al., 1983). The half-life of AII in vivo is substantially shorter than that in whole blood or plasma in vitro. These findings suggest that in vivo, AII is hydrolysed primarily by tissue angiotensinases (Biron et al., 1968; Ng and Vane, 1968; Leary and Ledingham, 1969; Osborne et al., 1970; Oparil and Bailie, 1973; Regoli et al., 1974; Ryan, 1974).

MODULATION OF ANGIOTENSIN METABOLISM

It has been suggested that the modulation of the rate of angiotensin metabolism is involved in the control of circulatory AII levels (Blair-West, 1976). Alterations in sodium balance have been reported to alter angiotensin metabolism, although the results of different workers do not agree. Lubash et al., (1971) and Johnston et al., (1972) found

that sodium depletion altered angiotensin metabolism in man while Nast et al., (1975) observed no change. Leary and Ledingham (1970a; 1970b) found that the capacity of the kidney, but not the liver, to catabolise AII was reduced by sodium loading, and enhanced by sodium depletion. However, Hollenberg et al., (1974) and more recently, Brooks et al., (1983) observed that the half-life of AII in the circulation was unaltered during sodium loading or restriction, and concluded that differences in sodium balance have no effect on the disappearance of AII from the circulation.

INTRARENAL LOCALISATION OF COMPONENTS OF THE RENIN-ANGIOTENSIN SYSTEM

As discussed in the previous sections, renin substrate, renin and converting enzyme have been localised within the kidney. Bailie et al., (1971) observed that AII concentrations in dog lymph were over 100 times greater than in plasma. Similarly, Mendelsohn (1976; 1979) found that AII immunoreactive material was present in rat kidney in concentrations which greatly exceeded that which could be accounted for by trapped blood. More recently using immunohistochemistry, Taugner and co-workers found renin-like and AII-like immunoreactivity to co-exist within the granulated epithelial cells of the afferent arterioles in rat kidney (Taugner et al., 1982a;1982b;1982c;1983). In addition, Rightsel, et al., (1982) reported that a monolayer cell culture of juxtaglomerular cells contains immunoreactivity of renin, converting enzyme and angiotensins I, II and III. Together, these findings support the concept of AII generation by an intrarenal renin- angiotensin system (Thurau and Mason, 1974; Taugner et al., 1982a;1982b). The question as to whether intrarenally formed AII is synthesised within the granulated epithelial cells (Celio and Inagami, 1981) or in the extracellular space and subsequently taken up by endocytosis (Taugner and Hackenthal, 1981) remains to be clarified.

It is unlikely that the AI released from the juxtaglomerular cells directly into the lumen of the terminal portion of the afferent arteriole could exert a significant effect on afferent arterioles via the intra-renal formation of AII, although recirculated angiotensin could act on the afferent arterioles. However, AII released into the lumen of the afferent arteriole could pass through the glomerulus, efferent arterioles and peritubular capillaries and thus exert its effect on these structures (Mendelsohn, 1979). Alternatively, AII could exert its influences on the afferent and efferent arterioles and

glomerular and peritubular capillaries if it was formed in the surrounding interstitial space (Thurau, 1974; Morgan and Davis, 1975; Morgan and Gillies, 1977). In addition, the molecular weights of AI and AII would allow their filtration through the glomerular membrane and into the proximal tubule. Converting enzyme has been shown to be present in the brush border of proximal tubular cells (Takada et al., 1982), while specific binding sites for AII have been found in renal brush border membranes (Brown and Douglas, 1982).

Although it is now generally accepted that AII can be formed both intrarenally and at extrarenal sites and that this agent can serve as a circulating vasoactive substance or as an intrarenal hormone, the relative importance of these routes of angiotensin formation in the regulation of renal function remains to be clarified.

EFFECTS OF ANGIOTENSIN II ON RENAL FUNCTION

The renal actions of AII as a circulating hormone have been assessed by evaluating the responses to systemic or intra-arterial infusions of the peptide, whereas the role of AII as an intrarenal hormone has been investigated by the inhibition of the formation of endogenous AII with converting enzyme inhibitors (ie, teprotide and captopril) or by using AII receptor antagonists (ie, saralasin).

EFFECTS OF AII ON URINARY SODIUM AND WATER EXCRETION

Intravenous infusion of angiotensin elicits dose-dependent effects on urinary sodium and water excretion in man, rabbit, dog, monkey and rat (Navar and Langford, 1974). Infusion of low sub-pressor doses (1-50 $ng.kg^{-1}.min^{-1}$) elicits a decrease in urine flow rate and urinary sodium excretion (Barraclough, 1965; Louis and Doyle, 1965; Barraclough et al., 1967; Malvin and Vander, 1967; Bonjour and Malvin, 1969; Johnson and Malvin, 1977).

Most investigators have attributed the observed antinatriuresis and antidiuresis to the associated marked decrease in renal blood flow (RBF) and lesser fall in glomerular filtration rate (GFR) (Bock et al., 1958; Peart, 1959b; De Bono et al., 1963; Louis and Doyle, 1965; Malvin and Vander, 1967; Bonjour and Malvin, 1969). Infusion of pressor doses of AII causes a transient decrease in urinary sodium excretion and urine flow rate followed by a marked natriuresis and diuresis (Barraclough, 1965; Healy et al., 1965; Barraclough et al., 1967; Malvin and Vander, 1967; Bonjour and Malvin, 1969; Healy and Elliott, 1971). The initial phase of the response to pressor doses of AII may be explained, as can the response to low doses, by the concomitant decrease in RBF and GFR. However, the mechanism responsible for the natriuretic phase of the response is still not clear. Several groups reported that the natriu-

retic response was not related to the pressor response and that the maximum natriuretic response occurred after blood pressure had fallen some way towards control level (Villarreal et al., 1964; Louis and Doyle, 1965). Healy and Elliott, (1971) showed that the natriuretic response in rabbits still occurred even when the increase in blood pressure was prevented by haemorrhage, and postulated that a direct inhibitory effect on tubular reabsorption by angiotensin was responsible for the natriuresis.

Although the response to infusion of low doses of AII has been explained by the secondary changes in haemodynamics, Barraclough et al., (1967) and Johnson and Malvin (1977) showed that the antinatriuretic response to infusion of a low dose of angiotensin was independent of changes in GFR, and suggested that angiotensin in small doses facilitates tubular sodium reasorption.

Barraclough et al., (1967) reported that the antinatriuretic response to low doses of AII in conscious rats was observed only when the animals had been chronically sodium loaded and that sodium depletion abolished or even converted the antinatriuretic effect of angiotensin to a natriuretic one. They suggested that in its normal state, the rat produces almost optimal antinatriuretic amounts of angiotensin in order to maintain sodium homeostasis and that it is necessary to sodium load the animal to suppress endogenous angiotensin production, in order to demonstrate an antinatriuretic effect of the infused hormone.

HAEMODYNAMIC EFFECTS

There is controversy regarding the major site at which AII regulates renal haemodynamics. Clearance studies in both intact animals and isolated perfused kidneys have demonstrated that AII elicits dose-dependent decreases in RBF and proportionately smaller decreases in

GFR such that filtration fraction increases (Navar and Langford, 1974; Davalos et al., 1978; Lohmeier and Cowley, 1979; Krahe et al., 1981; Rosivall and Navar, 1983). Because of the greater reductions in RBF than GFR it has been suggested that AII exerts a preferential effect on the efferent arterioles (Levens et al., 1981a; Hall, 1982). However, this is not necessarily the only explanation since increases in filtration fraction can occur even with balanced increases in pre- and postglomerular resistances (Rosivall and Navar, 1983). In micropuncture studies it has been observed that systemic or renal artery infusions of AII increase both afferent and efferent arteriolar resistances (Myers et al., 1975; Blantz et al., 1976; Rosivall and Navar, 1983). Further, Click et al., (1979) demonstrated that in the hamster renal transplant preparation both afferent and efferent arterioles responded directly to AII although the effects of the peptide appeared to be slightly greater on the efferent arteriole. These data indicate that AII exerts vasoconstrictor actions on both pre- and postglomerular resistance vessels.

It is possible that some of these effects on the afferent arteriole were not the result of the direct effects of the hormone but were indirect effects due to activation of other intrinsic autoregulatory mechanisms such as myogenic or tubuloglomerular feedback control. For example, Myers et al., (1975) found that although AII infusion raised both afferent and efferent arteriolar resistances, when the renal perfusion pressure was kept constant, the increase in the afferent but not the efferent resistance was almost abolished. Ichikawa et al., (1979) observed that only the efferent arteriolar resistance increased when AII was infused directly into the renal artery. More recently, Hall and Granger, (1983) found that when endogenous AII formation was blocked by captopril infusion and renal perfusion pressure was held constant, infusion of AII caused no changes in GFR, but reduced renal

blood flow and increased both pre- and postglomerular resistance. However, when glomerular filtration was stopped by ureteral occlusion, no change in preglomerular resistance was observed during infusion of AII. In a study using isolated rabbit renal microvessels, it was demonstrated that the efferent arteriole responded to AII (10^{-12} to 10^{-8}M) whereas the interlobular artery and afferent arteriole did not (Edwards, 1983). These results were interpreted as providing support for the concept that the primary site of action of AII is on the efferent arteriole.

However it is possible that the apparent lack of responsiveness of the preglomerular vessels to AII observed under some conditions is due to the counteracting effects of vasodilator prostaglandins. Indeed, Baylis and Brenner (1978) reported that the effects of AII, in particular on preglomerular resistance, were much more pronounced during inhibition of prostaglandin synthesis. These results are consistent with the findings that AII can stimulate intrarenal production of vasodilator prostaglandins (Brenner et al., 1980; McGiff, 1980; Schor et al., 1980; Baer, 1981), which, in turn, may help protect the renal circulation from the vasoconstrictor effects of AII. Thus, the actual resistance alterations observed in response to AII may be determined by the relative status of the intrarenal prostaglandin system.

EFFECTS ON Kf

In addition to it's reported actions on renal vascular resistance, AII has been reported to decrease the glomerular capillary ultrafiltration coefficient (Kf) (Myers et al., 1975; Blantz et al., 1976). More recently it was found that the Kf-reducing effects of other vasoactive hormones including prostaglandin E_2, prostacyclin and parathyroid hormone were abolished by the competitive AII antagonist, saralasin, suggesting that these substances exert their effects on Kf

via an increase in AII activity (Schor et al., 1981). These vasoactive hormones have also been shown to enhance cyclic nucleotide production (cAMP and/or cGMP) in mammals (Dousa et al., 1980). In addition, cAMP has been shown to be capable of stimulating renal renin release and thus AII production (Okahara et al., 1977; Hofbauer et al., 1978; Campbell et al., 1979; Freeman et al., 1984). Therefore, it has been suggested that these vasoactive hormones decrease Kf by virtue of their ability to stimulate renal renin release and thus AII production via the generation of cAMP, the resulting increase in local glomerular AII concentration causing mesangial cell contraction and a fall in Kf (Schor et al., 1981; Dworkin et al., 1983).

AII has been reported to decrease Kf in a variety of physiological conditions, including chronic sodium depletion (Steiner et al., 1979; Schor et al., 1980; Tucker and Blantz, 1983), chronic elevations in ureteral pressure (Ichikawa and Brenner, 1979), aortic constriction with reductions in renal artery pressure (Ichikawa and Brenner, 1980) and acute renal denervation (Pelayo and Blantz, 1984). These results suggest that AII influences glomerular function by directly modulating glomerular capillary ultrafiltration as well as by controlling pre- and postglomerular resistances. Although AII has been shown to contract mesangial cells in culture as well as glomeruli in vivo and in vitro, the exact mechanism whereby AII decreases Kf is not known (Hornych et al., 1972; Sraer et al., 1974; Hornych and Richet, 1977; Ausiello et al., 1980; Foidart et al., 1980; Mahieu et al., 1980).

EFFECTS ON THE PERITUBULAR CAPILLARIES

In microperfusion studies, it was demonstrated that AII infused directly into the peritubular capillaries contracted the capillaries and increased peritubular capillary and proximal tubular hydrostatic pressures (Jensen and Steven, 1977; Steven and Thorpe, 1977). These data

therefore indicate that AII may influence peritubular capillary dynamics through a direct effect on peritubular capillary structures.

EFFECTS ON INTRARENAL DISTRIBUTION OF BLOOD FLOW

The effects of AII on the regional distribution of RBF are controversial due to the different responses observed with different methods. Using the inert gas-washout technique, Carriere and Friborg (1969) and Rentsch et al., (1976) reported that AII was capable of causing a preferential decrease in cortical blood flow while maintaining or increasing relative blood flow through the deep juxtamedullary nephrons. It was suggested that this could explain the antidiuretic effect of angiotensin. However, using the hydrogen washout technique, Aukland, (1968) had previously found that angiotensin failed to produce a redistribution of renal blood flow, even when a wide range of doses were employed. Using radiolabelled microspheres, Rector et al., (1972) found that angiotensin infusions produced a parallel decrease in renal blood flow in each zone. Itskovitz and McGiff, (1974) observed a reduction in inner cortical microsphere distribution during AII infusions in isolated perfused dog kidneys, although this was found only to occur after prostaglandin production has been stopped with indomethacin. More recently Britton (1981) reported that renal artery infusion of both AI and AII produced greater decreases in inner cortical blood flow than outer cortical flow as determined by the distribution of radioactive microspheres. The differences between the results of these studies may be related to the dose or duration of infusion of AII employed in each study. Despite the different responses observed with different flow measurement methods, it can be concluded that all regions of the renal vasculature respond to exogenous AII, but subtle quantitative differences in regional sensitivity may exist.

EFFECTS ON RENIN RELEASE

Infusion of AII inhibits renal renin release (Vander and Geelhoed, 1965; Blair-West et al., 1971b; Shade et al., 1973; Davis and Freeman, 1976). Blair-West et al., (1971b) reported that physiological levels of AII acted directly on the kidney to decrease renin secretion in sheep and that the response was independent of changes in renal arterial pressure and sodium excretion. Shade et al., (1973) observed a decrease in renin secretion when small physiological amounts of AII were infused into the renal artery of a non-filtering kidney preparation. It has been observed that angiotensin decreases renin release in the absence of changes in sodium excretion rate and of a functional macula densa, in a non-filtering isolated perfused kidney preparation (Vandongen and Peart, 1974). Also, using the rat renal cortical slice preparation, Naftilan and Oparil have shown that AII inhibits renin release in a dose dependent manner via a direct action on the juxtaglomerular cells (Naftilan and Oparil, 1978; 1981; 1982). This negative feedback effect of AII on renal renin release appears to be dependent upon the presence of external calcium (Vandongen and Peart, 1974; Churchill, 1980; Naftilan and Oparil, 1982).

EFFECTS OF BLOCKERS OF THE RENIN-ANGIOTENSIN SYSTEM ON RENAL HAEMODYNAMICS

Pharmacological inhibitors or antagonists that block the formation or action of AII have been used to evaluate the intrarenal effects of AII. The responses observed with these blockers depend to a large extent on the pre-existing level of activity of the renin-angiotensin system (Regoli et al., 1974; Kimbrough et al., 1977; Fagard et al., 1978; Hollenberg, 1979; 1982; Wong and Zimmerman, 1982). In animals in which the activity of the renin-angiotensin system is normal or enhanced by dietary sodium restriction, angiotensin blockade is associated with a

small decrease in arterial blood pressure and marked increases in RBF and water and electrolyte excretion (Hall et al., 1977b; Kimbrough et al., 1977; Lohmeier et al., 1977; Fagard et al., 1978; Hollenberg, 1979; Navar et al., 1979; 1982; Ploth et al., 1979; Ploth and Roy, 1982a; Carmines et al., 1983). However, the effects on GFR have been less consistent; some studies have shown small increases (Kimbrough et al., 1977; Lohmeier et al., 1977; Ploth et al., 1979; Navar et al., 1982; Ploth and Roy, 1982a; Carmines et al., 1983) whereas others have failed to demonstrate significant changes (Fagard et al., 1978; Clappison et al., 1981).

Because the GFR responses to angiotensin blockade are less consistent and smaller in magnitude than the RBF responses, the data have been interpreted as indicating that AII exerts a preferential effect on the efferent arterioles (Hall et al., 1977b; 1977c; Johns, 1980; Levens et al., 1981a; Hall, 1982). In addition it has been suggested that intrarenally formed AII helps to maintain efferent arteriolar tone, particularly when renin secretion rate is high, such as during sodium restriction or reduced renal perfusion pressure (Hall et al., 1977c; Johns, 1980; Hall, 1982). This is based on the finding that GFR autoregulation, but not RBF autoregulation, is markedly attenuated in sodium-restricted animals infused with angiotensin blockers and in renin-depleted dogs (Hall et al., 1977a; 1977c; Johns, 1980; Hall, 1982). However, other studies have failed to demonstrate an impairment of GFR or RBF autoregulatory capability during infusions of angiotensin blockers (Gagnon et al., 1974; Anderson et al., 1975; Kaloyanides and Di Bona, 1976; Navar et al., 1982).

Navar et al., (1982) demonstrated that infusion of captopril in sodium-restricted dogs caused significant increases in RBF and GFR and a significant decrease in filtration fraction. Using micropuncture techniques they found that when renal perfusion pressure was kept

constant, captopril induced approximately equivalent decreases in afferent and efferent arteriolar resistances. Using sodium-restricted rats, Steiner et al., (1979) observed that administration of the AII antagonist saralasin caused an increase in single nephron glomerular filtration rate (SNGFR) and glomerular plasma flow and resulted in significant decreases in preglomerular resistance. Further, Ploth et al., (1979) reported that infusion of teprotide in hydropenic rats caused a significant increase in SNGFR. Therefore, the data from micropuncture studies utilising blockers of the renin-angiotensin system are consistent with the concept that the vasoconstrictor action of AII is exerted approximately equally by pre- and postglomerular vessels and do not support the notion that AII exerts a preferential effect on the efferent arteriole.

THE ROLE OF ANGIOTENSIN II IN THE TUBULOGLOMERULAR FEEDBACK MECHANISM

The tubuloglomerular feedback mechanism is responsive to flow related changes in distal tubular fluid composition such that increases in distal flow rate lead to increases in afferent arteriolar resistance and decreases in SNGFR (Guyton et al., 1964; Thurau, 1964; Wright and Briggs, 1979; Briggs and Wright, 1979; Navar et al., 1980; 1981; Bell and Navar, 1982; Schnermann, 1981). This mechanism operates at the single-nephron level and exerts a major controlling influence on GFR (Briggs and Wright, 1979; Wright and Briggs, 1979; Navar et al., 1980; 1981; Schnermann, 1981; Bell and Navar, 1982). The tubuloglomerular feedback mechanism is distinct from the phenomenon of glomerulotubular balance (GTB). GTB is defined as the ability of each successive segment of the proximal tubule to reabsorb a constant fraction of glomerular filtrate and solutes delivered to it. For a more detailed account of glomerulotubular balance the reader is referred to the following comprehensive review articles (Gertz and Boylan, 1973; Steven, 1977; Kiil, 1982; Haberle and Von Baeyer, 1983).

Thurau and co-workers postulated that increased concentrations of sodium chloride delivered to the macula densa area of the distal tubule result in increased local renin activation or release which in turn results in increased formation of AII and afferent arteriolar constriction (Thurau and Mason, 1974; Thurau, 1974; 1981; Thurau et al., 1982). This proposal that increased formation of AII is the mediator of feedback-induced changes in afferent arteriolar resistance and GFR is however at variance with the finding that increased renin release is associated with decreases in distal sodium chloride concentration (Vander, 1967; Davis and Freeman, 1976; Churchill et al., 1978). In addition, it has been demonstrated that most manipulations that increase

renin release such as sodium depletion, increased activity of sympathetic nervous system or reductions in arterial pressure are associated with decreases in distal sodium chloride concentration (Vander, 1967; Davis, 1973; Davis and Freeman, 1976). Furthermore, Muller-Suur et al., (1975) reported that in animals subjected to a wide variety of manoeuvres designed to alter intrarenal renin contents, feedback activity did not correlate with the measured renal renin contents directly. These data are therefore not consistent with the hypothesis that increased local formation of AII mediates the feedback responses to increases in distal flow rate.

It has been shown that pharmacological blockade of the renin-angiotensin system attenuates but does not abolish tubuloglomerular feedback activity (Ploth et al., 1979; Stowe et al., 1979; Ploth and Roy, 1982b). In addition, infusion of AII during converting enzyme blockade results in partial restoration of feedback activity (Ploth and Roy, 1982b). These results are consistent with the hypothesis that AII acts to modulate the sensitivity of the feedback mechanism rather than directly mediate the feedback-induced afferent arteriolar constriction (Navar et al., 1980; Bell and Navar, 1982; Ploth and Roy, 1982b).

EFFECTS OF ANGIOTENSIN III ON RENAL FUNCTION

Freeman et al., (1975) investigated the effects of intrarenal infusion of both AII and (des - 1 - Asp) AII (AIII) on renal function in normal and sodium-depleted dogs. In sodium-replete dogs, both peptides caused comparable reductions in RBF and renin secretion although no change in arterial pressure, creatinine clearance or sodium excretion was observed with either peptide. In sodium-depleted dogs, although both AII and AIII reduced renin secretion, neither peptide significantly altered RBF, GFR, blood pressure or sodium excretion, suggesting that AIII, like AII inhibits renal renin release.

Although it has been reported that AIII has only 25 - 50% of the pressor activity of AII, Freeman et al., (1975) and Caldicott et al., (1981) reported that AII and AIII were nearly equi-potent on the renal vasculature. However, Taub, et al., (1977) and Satoh et al., (1981) reported that in the dog, AII had a greater effect on the renal vasculature than AIII. Similarly, Hall et al., (1979a) found that intrarenal infusion of the heptapeptide antagonist (des - Asp^1, Ile^8) - AII in sodium-depleted dogs did not alter any aspect of renal function, indicating a minor physiological role for AIII in the control of renal function.

It has been reported that in both man and dog, a small proportion of the total circulating immunoreactive angiotensin is in the heptapeptide form, whereas the plasma of the rat contains approximately equal amounts of AII and AIII (Caravaggi et al., 1976; Semple et al., 1976; Semple and Morton, 1976), suggesting that in the rat, AIII may have a physiological role. Recently, Harris and Munro, (1984) compared the abilities of AII and AIII to reverse the natriuretic and diuretic effects of converting enzyme inhibition (teprotide) in anaesthetised rats. They found that systemic infusion of teprotide resulted in a slight decrease in arterial pressure and marked increases in urine flow

and sodium excretion. Both AII and AIII reversed the renal responses to teprotide, although AII was found to be more potent than AIII. These results suggest that in the rat, AIII may contibute to the regulation of renal sodium and water excretion.

EVIDENCE FOR A DIRECT TUBULAR ACTION OF ANGIOTENSIN II

Evidence for a direct effect of AII on tubular transport has been obtained from studies on the kidney in vivo and on isolated renal and non-renal tissues.

Vander (1963) using stop-flow experiments in dogs, showed that angiotensin infusion elevated the distal tubular concentrations of sodium and suggested that angiotensin might act to inhibit the distal tubule sodium transport mechanism. A non-specific pressor effect of the peptide was ruled out as equipressor doses of noradrenaline did not produce similar effects on the distal tubular sodium concentrations. Leyssac (1964; 1965) observed that infusion of AII increased proximal tubular occlusion time and suggested that AII inhibits proximal tubular sodium reabsorption. Healy and Elliott (1970) found that large doses of AII resulted in decreased medullary concentrations of sodium chloride and urea, suggesting that angiotensin has an effect on the ascending limb of the loop of Henle.

This suggestion of a direct action of the peptide at a site in the medulla was supported by a recent study in which angiotensin was shown to exert a primary antidiuretic action independent of changes in GFR or circulating vasopressin (Levens et al., 1981d). Further evidence for a direct effect of angiotensin on tubular transport was provided by Healy and Elliott (1971) who demonstrated that angiotensin-induced natriuresis in conscious rabbits occurred even when the increase in blood pressure was prevented by haemorrhage. In addition, Barraclough et al., (1967) showed that infusion of low doses of AII decreased urine flow and sodium excretion without significantly altering GFR. Similarly, Johnson and Malvin (1977) reported that low doses of AII markedly reduced urine flow and sodium excretion in the absence of changes in GFR. Although there were associated changes in renal plasma flow and filtration fraction,

these changes did not correlate with the observed excretory responses. While suggesting a direct effect of angiotensin on tubular reabsorption, these clearance studies involving infusion of angiotensin or converting enzyme inhibitors did not allow separation of the haemodynamic effects of the peptide from possible direct effects on transport.

MICROPUNCTURE STUDIES

Using in vivo stationary microperfusion techniques, Lowitz et al., (1969) found that peritubular capillary perfusion with high doses of AII (2×10^{-6} M) resulted in inhibition of distal tubular fluid reabsorption although no effect on proximal tubular reabsorption was observed. In contrast, using free-flow micropuncture techniques, Steven (1974) reported that proximal tubular fluid reabsorption was inhibited when the peritubular capillaries were perfused with 2×10^{-8}M AII.

Spinelli and Walther (1979) applied AII to the decapsulated cortical surface of the rat kidney and found stimulation of proximal tubular fluid reabsorption at 10^{-10}M, but inhibition at 10^{-5}M. Harris and Young (1977) investigated the effects of AII on the steady-state sodium concentration gradient (ΔC_{Na}) in rat proximal tubules using stationary microperfusion combined with perfusion of the peritubular capillaries. They found that high concentrations (10^{-6}M) inhibited, while low concentration (10^{-11}M) stimulated proximal ΔC_{Na}. In addition, they found no effect of angiotensin on trans-epithelial potential difference as measured by the steady-state distribution of chloride across the epithelium. These results suggest that angiotensin has a direct biphasic effect on proximal tubular sodium reasorption, although an effect due to changes in proximal tubular sodium permeability cannot be ruled out. More recently, Harris et al., (1984) found that acute converting enzyme blockade resulted in a reduced proximal tubular fluid reabsorption in both normotensive and two-kidney,

one-clip Goldblatt hypertensive rats.

Although it is possible that the reduced rates of tubular fluid reabsorption were due to the direct effects of altered renal haemodynamics and secondary alterations of the physical factors in the peritubular capillaries, these results support the possibility of a direct effect of AII on proximal tubular reabsorption and provide evidence that the peptide contributes to the maintenance of the rate of proximal fluid reabsorption.

STUDIES USING ISOLATED SODIUM-TRANSPORTING EPITHELIA

To avoid the complicating haemodynamic factors encountered in the intact kidney, the action of angiotensin on a variety of isolated sodium-transporting epithelia has been studied. However, the results of these studies have been confusing and appeared to depend on the type of epithelium and conditions employed.

Burg and Orloff (1968) found that high concentrations (2.5×10^{-6}M) of AII did not alter the rate of fluid absorption by isolated rabbit proximal tubules. Similarly, angiotensin did not affect sodium transport by toad skin (Barbour et al., 1964), toad bladder (Coviello and Crabbe, 1965) or isolated kidney cortex slices (Healy and Douglas, 1968). In contrast to these findings, McAfee and Locke (1967) reported that angiotensin stimulated sodium transport by a frog-skin preparation, whereas angiotensin was found to inhibit isotonic absorption of salt and water by rabbit gall bladder in vitro (Leyssac et al., 1974). Further evidence for a direct effect of angiotensin on epithelial transport was provided by the study of Healy et al., (1976) in which physiological doses of the peptide (10^{-11} to 10^{-9}M) were found to inhibit sodium transport in the main duct of the rabbit mandibular gland in vitro. In addition, Crocker and Munday (1967) found that angiotensin stimulated fluid transfer by isolated everted sacs prepared from the jejunum of

adrenalectomised and nephrectomised rats. This finding was supported by the finding that the small intestine in vivo and the isolated large and small intestines respond to AII with net absorption of sodium and water at low doses (10^{-12} - 10^{-11}M) and an inhibition of sodium and water transport at high doses (10^{-9} - 10^{-8}M) (Davies et al., 1970; Bolton et al., 1975). Munday et al., (1971) reported that low doses (10^{-12}M) of AII increased the rate of active sodium extrusion by rat kidney cortex slices. This effect did not involve cyclic AMP, but was blocked by inhibitors of the translation stage of protein synthesis (Munday et al., 1972). Further evidence for a direct effect of angiotensin on renal tubular sodium transport was provided by Simmons (1978) using monolayer cultures of dog kidney cells (MDCK cell-line). He showed that net sodium transport was stimulated by low concentrations of AII (10^{-11}M). Similar findings were reported by Simpson and Goodfriend (1984) who found that low concentrations of AII (10^{-10} and 10^{-9}M) stimulated $^{22}Na^{+}$ efflux from cultured proximal tubule cells. In addition, Schuster et al., (1984) demonstrated that low concentrations (10^{-11}M) of angiotensin stimulated, while high concentrations (10^{-7}M) inhibited, fluid reabsorption by the isolated rabbit proximal tubule and concluded that angiotensin exerts a direct biphasic effect on proximal tubular fluid reabsorption.

These studies provide evidence that angiotensin exerts a direct action on transepithelial sodium and fluid transport, either inhibitory or stimulatory, depending on the hormone concentration and the properties of the epithelium used.

RENAL TUBULAR ANGIOTENSIN II RECEPTORS

Freedlender and Goodfriend (1977) demonstrated the presence of two classes of specific (^{125}I) AII-binding sites on isolated rat renal tubular fragments. The concentrations of the binding sites varied as a

function of sodium balance and binding of AII (10^{-12}M) resulted in increased extrusion of $^{22}Na^{+}$ from the tubules. More recently Brown and Douglas (1982; 1983) identified specific (^{125}I) AII-binding sites on isolated rat renal brush border membranes and in basolateral cell membranes isolated from rat and baboon renal cortical tissue. The affinity of the brush border binding sites was found to be approximately 3-fold lower than that of the basolateral membrane binding sites.

The presence of specific binding sites for angiotensin on renal tubules supports the finding that angiotensin exerts a direct effect on proximal tubular transport. Also, the presence of two distinct binding sites on the same epithelial cell (Brown and Douglas, 1983) suggests that there may be more than one mechanism whereby angiotensin influences tubular transport.

MECHANISM OF TUBULAR ACTION

Although there is evidence for a direct effect of AII on tubular reabsorption, the mechanism underlying both the stimulatory and inhibitory responses to the peptide remain unclear. Stimulation of net sodium transport across frog-skin was associated with an increase in short circuit current (McAfee and Locke, 1967), while the reductions in net reabsorption of sodium by the isolated rabbit mandibular gland (Healy et al., 1976) were linked to depolarisations of the trans-epithelial p.d., suggesting that in these tissues, angiotensin influences epithelial transport by electrogenic mechansims.

In contrast, the action of AII on epithelial transport in the small intestine in vivo, the colon in vivo, the gallbladder in vitro, the proximal tubule in vivo and the isolated rabbit proximal convoluted tubule occurred in the absence of changes in electrical potential difference, suggesting that in these tissues, angiotensin alters epithelial transport via electoneutral mechanisms (Leyssac et al., 1974;

Bolton et al., 1975; Harris and Young, 1977; Levens et al., 1981b; Schuster et al., 1984).

Recently it was shown that stimulation of water absorption from the rat jejunum by AII was mediated by release of noradrenaline from sympathetic nerve endings in close proximity to the transporting epithelial cells (Levens et al., 1979). In addition, Levens et al., (1981c) found that meclofenamate and indomethacin abolished the inhibition of jejunal water transport produced by high concentrations of AII, and suggested that the response of the rat jejunum to high doses of AII may be mediated by prostaglandins. Therefore, it is possible that as for the rat jejunum, the effect of low doses of AII on proximal tubular reabsorption may be mediated by noradrenaline released from adrenergic nerve endings. Indeed, Bello-Reuss (1980) demonstrated that fluid absorption by the isolated rabbit proximal convoluted tubule was stimulated by high concentrations of noradrenaline (10^{-6}M). This finding provides direct evidence for an effect of catecholamines on the rate of fluid transport by the proximal convoluted tubule. Thus, it is possible that noradrenaline release might be a mediating step in the action of AII. Indeed, Radhi et al., (1982) found that in the rat the α_1-receptor antagonist prazosin, blocked the antidiuretic and antinatriuretic effects of low doses (20 and 50 ng.kg.$^{-1}$min^{-1}) of AII, but not the diuretic or natriuretic effects of high doses (700 ng.kg.$^{-1}$min^{-1}) indicating that there is some involvement of the sympathetic nervous system in the renal response to low doses of AII, but not in the response to high doses of the peptide.

However, angiotensin has been shown to have a stimulatory effect on sodium efflux from cultured kidney tubules (Simpson and Goodfriend, 1984), a stimulatory effect on net sodium transport in cultured MDCK cell monolayers (Simmons, 1978) and a biphasic effect on isolated proximal tubular reabsorption (Schuster et al., 1984). As it is likely

that these preparations were devoid of adrenergic nerve connections, it would appear that AII can exert a biphasic effect on proximal tubular reabsorption independent of the sympathetic nervous system. Such a direct effect is probably mediated by binding of the peptide to the specific binding sites on the basolateral membrane. The intracellular mechanism whereby AII affects tubular reabsorption remains unknown, although it is possible that the peptide may act via changes in intracellular cyclic AMP levels and/or cytosolic calcium concentration (Peach, 1981;Harris and Navar, 1985).

DRIVING FORCES FOR FLUID ABSORPTION BY THE PROXIMAL TUBULE

The reabsorption of fluid and solute by the proximal tubule involves the complex interaction of active and passive processes across the tubular epithelium and uptake of the reabsorbate by the peritubular capillaries. Sodium diffuses down its electrochemical concentration gradient from tubule lumen into the cell, and is then actively transported into the interstitial space, with water following passively. Uptake of this reabsorbate from the interstitial space into the peritubular capillary is determined by the balance of Starling forces across the capillary wall.

HISTORICAL PERSPECTIVE

It has been generally accepted that volume absorption in leaky epithelia ie, proximal tubule or small intestine, is an isotonic process. Walker et al., (1941) compared the relative osmolalities of proximal tubular fluid and plasma and found the tubular fluid to be slightly hypotonic to plasma. However, as the relative osmolality differences were small they concluded that the tubule contents during and after reabsorption of large amounts of fluid remained in osmotic equilibrium with plasma. In addition, Gottschalk and Mylle (1959) reported that proximal tubular fluid samples were isotonic to venous plasma under a variety of conditions. Furthermore, it was demonstrated that the osmolality of the luminal fluid did not change during volume absorption in either the proximal convoluted tubule (Kokko et al., 1971) or the proximal straight tubule (Schafer et al., 1974).

Curran and MacIntosh (1962) demonstrated that osmotic volume flow could occur between two isosmotic solutions if they were separated by a central compartment bounded by membranes of differing permeability and

suggested that the presence of a hypertonic central compartment within epithelia might be responsible for apparent isotonic absorption. Diamond and Bossert (1967) then modified the Curran and MacIntosh hypothesis by proposing that active solute transport from luminal solutions into unstirred intercellular spaces might raise the osmolality of the latter thereby providing a driving force for transcellular water flow.

Recently however, evidence has been provided which suggests that the standing gradient hypothesis of Diamond and Bossert may either not apply and/or be inadequate to account for isotonic fluid absorption in the proximal tubule. The diffusional resistance of the paracellular pathway in the proximal tubule is very low and it has been suggested that this lateral intercellular space provides insufficient resistance to solute diffusion to permit the generation of significant osmotic gradients and that the interspace may be in diffusional equilibrium with peritubular solutions (Lutz et al., 1973; Schafer et al., 1975; 1977; Andreoli and Schafer, 1979a; 1979b). The hydraulic conductance of the proximal tubular epithelium was estimated by Schafer and Andreoli to be in the range of 3,000 - 4,000 $\mu m.sec^{-1}$, a value much greater than anticipated by the standing gradient theory, suggesting that very small osmotic gradients between luminal and peritubular solutions can drive proximal tubular absorption (Andreoli et al.,1978; Schafer et al., 1978b; Andreoli and Schafer, 1979a; 1979b). Finally, the prediction of intercellular space hypertonicity in the standing gradient hypothesis depends in part on the assumption that active solute transport is confined to the apical regions (Diamond and Bossert, 1967). However, Kyte (1976) showed that (Na^+,K^+)-ATPase activity was uniformly distributed along the paracellular pathway. If this activity is associated with the sites for active Na^+ transport into the intercellular spaces,

then it is likely that the net diffusion resistance for solute movement from intercellular spaces to interstitium is less than that proposed by the standing gradient theory.

EXTERNAL SOLUTION DRIVING FORCES

As a result of these findings it was proposed that external solution driving forces for net volume absorption might account for fluid absorption by the proximal tubule (Andreoli and Schafer, 1978; 1979a; 1979b). Indeed, given the high transepithelial conductance of the proximal tubule it was calculated that relatively small osmotic gradients between luminal and peritubular fluids could provide the driving force for the rates of fluid absorption observed in vivo and in vitro, and it was suggested that a small degree of effective luminal hypotonicity might drive proximal tubular fluid transport (Andreoli and Schafer, 1978; 1979a; 1979b).

Two separate, but not mutually exclusive mechanisms were proposed to account for the development of such effective transepithelial osmolality gradients. First, the generation of axial anion asymmetry between luminal and peritubular solutions. Second, the development of trivial degrees of luminal hypotonicity as a consequence of salt absorption.

AXIAL ANION ASYMMETRY

Proximal tubular sodium reabsorption is accompanied by tubular fluid acidification which results in the preferential absorption of HCO_3^- and an increase in the tubular fluid to peritubular solution Cl^- concentration ratio (Schafer et al., 1974; Neumann and Rector, 1976; Schafer and Andreoli, 1976; Warnock and Burg, 1977). The development of a rising luminal Cl^- concentration and a falling HCO_3^- concentration

EX BIBL. UNIV. EDINBURGH

along the proximal tubule produces an osmotic driving force for fluid absorption if the reflection coefficient for HCO_3^- exceeds that for Cl^-, even when luminal and peritubular fluid osmolalities are identical (Schafer et al., 1975; 1977; Andreoli and Schafer, 1979a; 1979b).

The possibility that axial anion asymmetry provides the driving force for isotonic fluid absorption was examined by Schafer et al., (1975; 1977). Using isolated rabbit proximal straight tubules, perfused with a Cl^- Krebs-Ringer (KR) solution (HCO_3^- = 3.6 mM, Cl^- = 138 mM) and bathed with a HCO_3^--KR solution (HCO_3^-=25mM; Cl^- = 113 mM), they found that fluid absorption was inhibited by approximately 60% by either cooling to 21°C or by addition of 0.1 mM ouabain to the bathing medium. In the absence of anion concentration gradients, ie, when both perfusate and bathing solutions were identical, either ouabain or cooling reduced fluid absorption to zero. These results suggest that fluid absorption from tubules perfused with Cl^--KR solution and bathed with HCO_3^--KR solution is dependent upon active sodium chloride absorption, which accounts for about two-thirds of the fluid absorption and passive sodium chloride absorption driven by a favourable electrochemical gradient for NaCl, accounting for about one-third of fluid absorption in the pars recta.

Chloride is absorbed passively down its concentration gradient. Since the permeability to chloride exceeds that to bicarbonate at least in proximal tubules of superficial nephrons (Fromter et al., 1973; Berry et al., 1978; Holmberg et al., 1981), a chloride bicarbonate bi-ionic potential develops, oriented lumen positive (Fromter et al., 1973; Kokko, 1973; Barratt et al., 1974; Schafer et al., 1974; Berry et al., 1978). This positive voltage drives passive Na^+ absorption. The passive component of NaCl absorption presumably occurs through the low resistance paracellular pathway. There may also be passive NaCl absorption due to solvent drag, although the extent of this remains

controversial (Fromter et al., 1973; Andreoli et al., 1979; Jacobsen et al., 1982).

Thus, an effective osmotic driving force for fluid absorption is generated as a consequence of the axial anion asymmetry resulting from preferential HCO_3^- absorption if the reflection coefficient for HCO_3^- exceeds that for Cl^- (Schafer et al., 1975; 1977; Andreoli and Schafer, 1979a; 1979b). In this respect, most early studies showed that the reflection coefficient of $NaHCO_3$ exceeded that for NaCl (Kokko et al., 1971; Fromter et al., 1973; Schafer et al., 1975). In contrast, Hierholzer et al., (1980) reported that the reflection coefficients for $NaHCO_3$ and NaCl were approximately equal. However, a more recent study showed that the reflection coefficient for $NaHCO_3$ was slightly greater than that for NaCl (Green and Giebisch, 1984).

This mechanism of axial anion asymmetry is not restricted to $NaHCO_3$ and NaCl but applies to any solute preferentially absorbed in the early regions of the proximal tubule, provided that the reflection coefficient of that solute is relatively high compared with the solutes remaining in the tubular fluid. Indeed, it has been suggested that preferential absorption of acetate, glucose and amino acids, which have reflection coefficients greater than that for sodium chloride or sodium bicarbonate produces an effective osmotic driving force for fluid absorption (Neumann and Rector, 1976; Schafer and Andreoli, 1976; Andreoli and Schafer, 1979a; 1979b; Hierholzer et al., 1980; Green and Giebisch, 1984).

LUMINAL HYPOTONICITY

To determine whether slight luminal hypotonicity could provide an osmotic driving force for proximal tubular fluid absorption, Schafer et al., (1978a) measured the spontaneous rates of fluid absorption occurring in isolated proximal straight tubules perfused with

symmetrical sodium chloride solutions lacking HCO_3^-. They found that net fluid absorption was about 0.2 $nl.mm^{-1}.min^{-1}$ and that net fluid absorption was inhibited by cooling or ouabain but was unaffected by the carbonic anhydrase inhibitor ethoxzolamide. From these results it was concluded that fluid absorption depended on "simple" active sodium absorption and that the associated change in transepithelial potential difference to lumen negative values, was the major driving force for passive Cl^- absorption (Andreoli and Schafer, 1978; Schafer et al., 1978 a). However, in contrast to this rheogenic sodium transport providing the driving force for passive paracellular Cl^- absorption in the straight segment of the proximal tubule, the active component of chloride absorption across the superficial and mid-cortical proximal convoluted tubules is neutral and transcellular (Berry and Rector, 1980). This process generates no transepithelial P.D. and can be blocked by anion transport inhibitors (eg, SITS, furosemide). Indeed, there is little evidence of simple rheogenic sodium transport capable of driving passive chloride absorption through paracellular shunt pathways in the convoluted segment of the proximal tubule.

Andreoli and Schafer (1978) calculated that due to the high hydraulic conductance of the epithelium, a fall in the luminal NaCl concentration of only 0.42-0.60 mM (undetectable by current methodology) would be required to account for the rate of fluid absorption (0.20 $nl.mm^{-1}.min^{-1}$) observed in isolated proximal straight tubules perfused and bathed with symmetrical NaCl solutions. Such a decrease in luminal NaCl concentration represented a fall in luminal osmolality of 0.65 mosmol/ $kg.H_2O$, (ie, a 0.22% decrease in luminal osmolality). Therefore, they concluded that a slight luminal hypotonicity would develop as a result of active sodium and passive chloride absorption, thereby providing a driving force for fluid transport across the epithelial wall and that a

steady state equilibrium is achieved between luminal and bathing solutions when fluid absorption occurs.

There is evidence for the existence of such a mechanism in the in vivo proximal tubule. Bishop et al., (1978) found that the osmolality of proximal luminal fluid was significantly lower than that of systemic plasma. More recently, Green and Giebisch (1984) found that the in vivo proximal tubule could generate a small cryoscopic luminal hypotonicity when both the tubule and peritubular capillaries were perfused with sodium chloride solutions, although when HCO_3^- was added to the peritubular capillary perfusate, no luminal hypotonicity was generated, even though fluid reabsorption was increased. In addition, using free-flow micropuncture techniques in rats, Liu et al., (1984) observed that luminal hypotonicity developed along the length of the proximal convoluted tubule. Together, these results indicate that in the rat proximal convoluted tubule, luminal hypotonicity and reabsorbate hypertonicity does occur.

DOMINANCE OF AXIAL ANION ASYMMETRY

The relative contributions of axial anion asymmetry and luminal hypotonicity to proximal tubular fluid absorption were determined using isolated proximal straight tubules perfused and bathed with symmetrical HCO_3^- - KR solutions (114 mM Cl^- and 25 mM HCO_3^-) (Schafer and Andreoli, 1976). Under these conditions, (ie, when tubules were exposed to solutions resembling glomerular ultrafiltrate and peritubular fluid) the net rate of fluid absorption was 0.46 $nl.mm^{-1}.min^{-1}$, and the mean intratubular Cl^- concentration increased to 122.1mM. From these findings and previously determined values for the hydraulic conductance and ionic reflection coefficients, Andreoli and Schafer (1979a; 1979b) calculated that approximately 90% of the effective osmotic driving force

for fluid transport in the proximal straight-tubule was referable to the tubular fluid Cl^-/HCO_3^- concentration ratio, and that the remainder of the driving force for fluid transport was due to a 0.2 mM reduction in the tubular fluid concentration. Thus the development of axial anion asymmetry is the dominant driving force for proximal tubular fluid absorption. Similarly, Green and Giebisch (1984) suggested that although the in vivo proximal tubule has the ability to generate a luminal hypotonicity, normally the effective osmotic gradients depend largely on preferential reabsorption of solutes in the early part of the tubule (ie, development of axial solute asymmetry).

Thus it would appear that the development of axial solute asymmetry due to preferential organic anion absorption, preferential non-electrolyte absorption and tubular acidification is the major driving force for fluid absorption in the in vivo mammalian proximal tubule (Andreoli and Schafer, 1979a; 1979b; Green and Giebisch, 1984).

ROLE OF PERITUBULAR STARLING FORCES IN THE REGULATION OF PROXIMAL TUBULAR FLUID REABSORPTION

In the proximal tubule in vivo, solute and fluid are transported from tubular lumen to the interstitial space by the mechanisms described in the previous section. This represents the first step in the reabsorption process. The second step involves uptake of the reabsorbate from the interstitial space into the peritubular capillaries. Uptake of reabsorbate into the peritubular capillaries is determined by the balance of Starling forces across the capillary wall.

Evidence has been provided both in support of and against the hypothesis that peritubular physical forces play an important role in the control of proximal tubular fluid reabsorption. Using micropuncture techniques, Lewy and Windhager (1968) found that increasing peritubular capillary hydrostatic pressure by partial constriction of the renal vein resulted in a decrease in the proximal tubular transport rate. This finding has been confirmed in both hydropenic and volume expanded rats (Sato, 1975; Burnett et al., 1982). Using free-flow micropuncture and microperfusion techniques, it was demonstrated that the absolute rate of proximal reabsorption varied directly with the oncotic pressure in the efferent arterioles (Brenner et al., 1969; Brenner and Troy, 1971). In addition, a direct relationship between efferent arteriolar protein concentration and proximal saline reabsorption was reported by Weinman et al., (1971). These results, together with findings of Spitzer and Windhager (1970) that the rate of proximal fluid reabsorption correlates with the colloid concentration in the peritubular capillaries support the hypothesis that peritubular capillary physical forces play an important role in the control of proximal tubular reabsorption, ie, that an increase in oncotic pressure or a decrease in hydrostatic pressure in

the peritubular capillaries will act to increase the rate of proximal reabsorption.

However, several studies have failed to find a relationship between proximal reabsorption and peritubular physical forces. Indeed several groups reported that perfusion of the peritubular capillaries with colloid-free solutions did not affect the rate of proximal reabsorption as compared with normal blood perfusion or with the addition of albumin to the perfusate (Rumrich and Ullrich, 1968; Baldamus et al., 1969; Lowitz et al., 1969). Similarly, Bank et al., (1972) found that microperfusion rate, but not the peritubular capillary oncotic pressure affected proximal tubular reabsorption. In addition, Conger et al., (1976) found that perfusion of the peritubular capillaries with colloid free solutions or hyperoncotic plasma did not alter the absolute rate of proximal reabsorption as compared with normal blood perfusion. Furthermore, it was found that proximal tubular reabsorption was unchanged during systemic infusion of the vasodilators acetylcholine, bradykinin and prostaglandin E_1 in rats, despite marked decreases in oncotic pressure and increases in hydrostatic pressure in the peritubular capillaries (Bayliss et al., 1976).

Ott et al., (1975) investigated the effect of increased peritubular oncotic pressure on proximal tubular reabsorption in both hydropenic and volume expanded dogs. They found that increases in peritubular capillary oncotic pressure increased proximal reabsorption in the volume expanded dogs, but not in the hydropenic animals and suggested that volume expansion alters the effect of increased peritubular oncotic pressure on proximal tubular reabsorption. These results are consistent with the view that extracellular volume expansion increases passive backflux. According to this view, a reduction in colloid osmotic pressure or an increase in hydraulic pressure in the peritubular capillaries would reduce uptake of fluid from the interstitial and intercel-

lular spaces and increase interstitial pressure. The lateral interspace would dilate and mechanically increase the permeability of the tight junctional complex. An increased fraction of the transported solute would leak back into the lumen and net solute and water reabsorption would be reduced.

Several studies have shown that during conditions of reduced proximal reabsorption (ie, during volume expansion or increased renal venous pressure) the permeability of the tight junctional complexes as measured by electrical conductivity or permeability to large solutes (ie, raffinose or sucrose) is increased (Boulpaep, 1972; Seely, 1973; Grandchamp and Boulpaep, 1974). Therefore it is possible that increases in the permeability of the tight junctional complexes and increased backflux are a prerequisite for increased peritubular oncotic pressure to result in increases in proximal reabsorption.

However, an increase in tight junction permeability would cause increased backleak only of those solutes whose concentrations in the luminal fluid fall below their respective plasma concentrations. Thus an increased tight junction permeability would be expected to inhibit reabsorption of glucose, amino acids and $NaHCO_3$ but actually enhance NaCl reabsorption because the electrochemical gradient for Na^+ and Cl^- along the proximal convoluted tubule favours absorption. However, it was found that extracellular volume expansion inhibited NaCl and volume absorption in the proximal tubule perfused in vivo with a high chloride and low bicarbonate solution (Alpern and Rector, 1983). As the orientation of the NaCl concentration gradient favoured passive absorption, the effect of volume expansion on NaCl and fluid absorption could not have been due to increased tight junction permeability, and therefore must have been due to inhibition of active transcellular NaCl transport. The mechanism whereby volume expansion could inhibit transcellular NaCl transport remains unclear.

Studies using isolated perfused proximal tubules have demonstrated that removal of protein from the bathing solution results in inhibition of fluid absorption without any associated changes in transepithelial P.D. or paracellular permeability (Imai and Kokko, 1972; 1974; Berry and Cogan, 1981; LaPointe et al., 1983). In addition, removal of protein from the bathing solution was found to inhibit only NaCl absorption by the isolated proximal tubule, and not glucose or $NaHCO_3$ absorption (Berry and Cogan, 1981). Furthermore, Berry (1983) demonstrated that removal of protein from the bathing solution resulted in a 60% reduction in volume absorption by the isolated proximal tubule. Cooling the tubule to 20°C was found to have no further effect on net fluid absorption, while reversing the procedure, cooling first followed by removal of bath protein, was found to give similar results. These results suggest that removal of protein from the bathing solution inhibits NaCl and fluid absorption by the isolated proximal tubule by inhibiting active neutral transcellular NaCl transport.

Although the role of physical factors in the regulation of proximal tubular reabsorption is controversial, it appears that peritubular Starling forces do affect proximal reabsorption under certain conditions, ie, during changes in extracellular volume. Indeed, it has been suggested that altered intrarenal physical forces play an important role in mediating the decrease in proximal reabsorption during extracellular volume expansion, and that the increase in urinary sodium and water excretion observed during volume expansion is due largely to the decreased rate of reabsorption by this segment of the nephron (Landwehr et al., 1967; Bank, 1970; Knox and Haas, 1982). The effects of peritubular Starling forces on proximal reabsorption appear to be mediated by an increase in the permeability of the tight junctional complex and thus an increase in passive backflux of those solutes whose tubular fluid concentration falls below their plasma level (ie, glucose,

amino acids and HCO_3^-), and by inhibition of active neutral transcellular NaCl transport.

In addition to intrarenal physical forces (peritubular Starling forces), several factors appear to play a role in the physiological control of proximal tubular fluid reabsorption. These include; the filtered load of specific solutes, the renal nerves and various hormonal factors (angiotensin II, catecholamines, natriuretic hormone, and prostaglandins). The interaction between these factors and their relative contribution to the control of proximal reabsorption has yet to be established.

PROPOSAL

As discussed in the literature review, AII administration has been shown to elicit dose-dependent effects on renal excretory function. Most studies have indicated that the antinatriuretic and antidiuretic effects of AII occur when low doses (1-50 $ng.kg.min^{-1}$) are administered, while infusion of larger doses may lead to a diuresis and natriuresis. Since there are usually associated reductions in renal blood flow and glomerular filtration rate, the antinatriuretic effects of AII could be the consequence of primary haemodynamic effects (Navar and Langford, 1974). However, several studies have suggested that this explanation may not fully account for the effects of AII on sodium excretion. Barraclough et al., (1967) reported that infusion of low doses of AII in conscious rats resulted in significant reductions in urine flow and urinary sodium excretion in the absence of changes in glomerular filtration rate. In addition, Johnson and Malvin, (1977) showed that reductions in urine flow and sodium excretion during intra-arterial infusions of low doses of AII did not correlate with changes in GFR, renal plasma flow or filtration fraction. These findings suggest that the antinatriuretic effects of AII may be due to a direct stimulatory action of the peptide on tubular sodium reabsorption.

An action of AII on tubular reabsorption has also been postulated to account for the natriuresis observed during infusion of pressor doses of the peptide. Indeed, Healy and Elliott, (1971) observed that in conscious rabbits, angiotensin induced natriuresis still occurred even when the increase in blood pressure was prevented by haemorrhage. They suggested that angiotensin-induced natriuresis is due to a direct inhibitory action of the peptide on tubular reabsorption.

Harris and Young, (1977) provided micropuncture evidence indicating that AII exerts a direct effect on proximal tubular sodium reabsorption.

Using stationary microperfusion techniques they demonstrated that low concentrations (10^{-11}M) stimulated, while higher concentrations (10^{-6}M) inhibited, proximal tubular sodium reabsorption. Similar findings were reported in a study using isolated perfused proximal convoluted tubules (Schuster et al., 1984). These studies provided evidence that angiotensin exerts a direct action on proximal tubular sodium and fluid reabsorption.

However, it is not known whether systemic infusion of AII results in alterations in proximal tubular reabsorption or whether such changes are involved in mediating AII induced alterations in renal excretory function. Thus, the purpose of the present study was to evaluate the effects of systemic infusion of AII on proximal tubular fluid reabsorption and whole kidney function in anaesthetised rats, and to assess the possible mechanism(s) whereby AII alters renal excretory function.

SECTION 2

METHODS

METHODS

ANIMALS

All experiments were performed on adult male Wistar rats maintained on a standard rat diet.

SURGICAL PROCEDURE

Rats were anaesthetised with a single I.P. injection of Inactin (International Apotheke, Hamburg, West Germany) (120 $mg.kg^{-1}$) and placed on a heated micropuncture table, thermostatically controlled to maintain their rectal temperature at 37°C. A tracheostomy was performed and the left carotid artery cannulated to allow blood sampling and continuous monitoring of arterial blood pressure. Mean arterial blood pressure (MABP) was monitored with a Bell and Howell pressure transducer (Type 4-422) and recorded on a servoscribe pen recorder. Two cannulae were inserted into the left jugular vein for infusion of 0.9% saline, inulin and drugs. The venous cannulae were attached to syringes containing 0.9% saline and operated by a continuous infusion pump (Braun).

The left kidney was prepared for micropuncture using a method similar to that described by Andreucci (1978a). Briefly, the rat was placed on its right side and a subcostal (transverse) incision made in the left flank of the rat's abdomen. The flank incision was made using a cautery unit. The abdominal wall was retracted using four brass pins and the abdominal contents covered with paraffin oil to minimise evaporative losses during surgery. The left kidney was freed from the surrounding tissue and immobilised in a plastic cup containing cotton wool padding. Care was taken not to damage the renal artery or vein. The kidney was then bathed continuously with paraffin oil preheated to 37°C to prevent it from cooling and drying out. The left ureter was cannulated with pp 10 tubing (Portex), and urine collected

under paraffin oil in pre-weighed tubes (2·2 ml Eppendorf).

The positions of the various cannulae are shown in Fig. 2.

EXPERIMENTAL PROTOCOLS

The experiments performed are reported in Sections 3-6.

The experiments reported in Section 3 were designed to evaluate the renal effects of infusion of a pressor dose of AII and to determine the mechanism of angiotensin induced natriuresis. Two groups of rats were used. In both groups, AII was infused at a rate of 100 $ng \cdot min^{-1}$ for one hour. In one group, the renal perfusion pressure of the left kidney was permitted to increase as the systemic arterial pressure increased during AII infusion, whereas in the other group, renal perfusion pressure of the left kidney was maintained at the control level by tightening a snare placed around the aorta proximal to the origin of the left renal artery. Thus, the effects of a pressor dose of AII on renal function were assessed both in the presence and absence of the accompanying increase in renal perfusion pressure.

The purpose of the experiments reported in Section 4 was to assess the effects of systemic infusion of low (sub-pressor) doses of AII on proximal tubular fluid reabsorption and whole kidney function. Three groups of experiments were performed. In one group, AII was infused at a rate of 0.1 $ng.min^{-1}$ for one hour. In a second group, AII was infused at successive rates of 1, 5 and 10 $ng.min^{-1}$ each for one hour. A third group of rats were infused with only 0.9% NaCl for a period of five hours and served as time controls.

Only subtle antidiuretic and antinatriuretic effects of AII were observed in the experiments reported in Section 4. These findings were interpreted as being consistent with the possibility that it may be necessary to suppress endogenous angiotensin production in order to demonstrate a pronounced antinatriuretic effect of infused hormone.

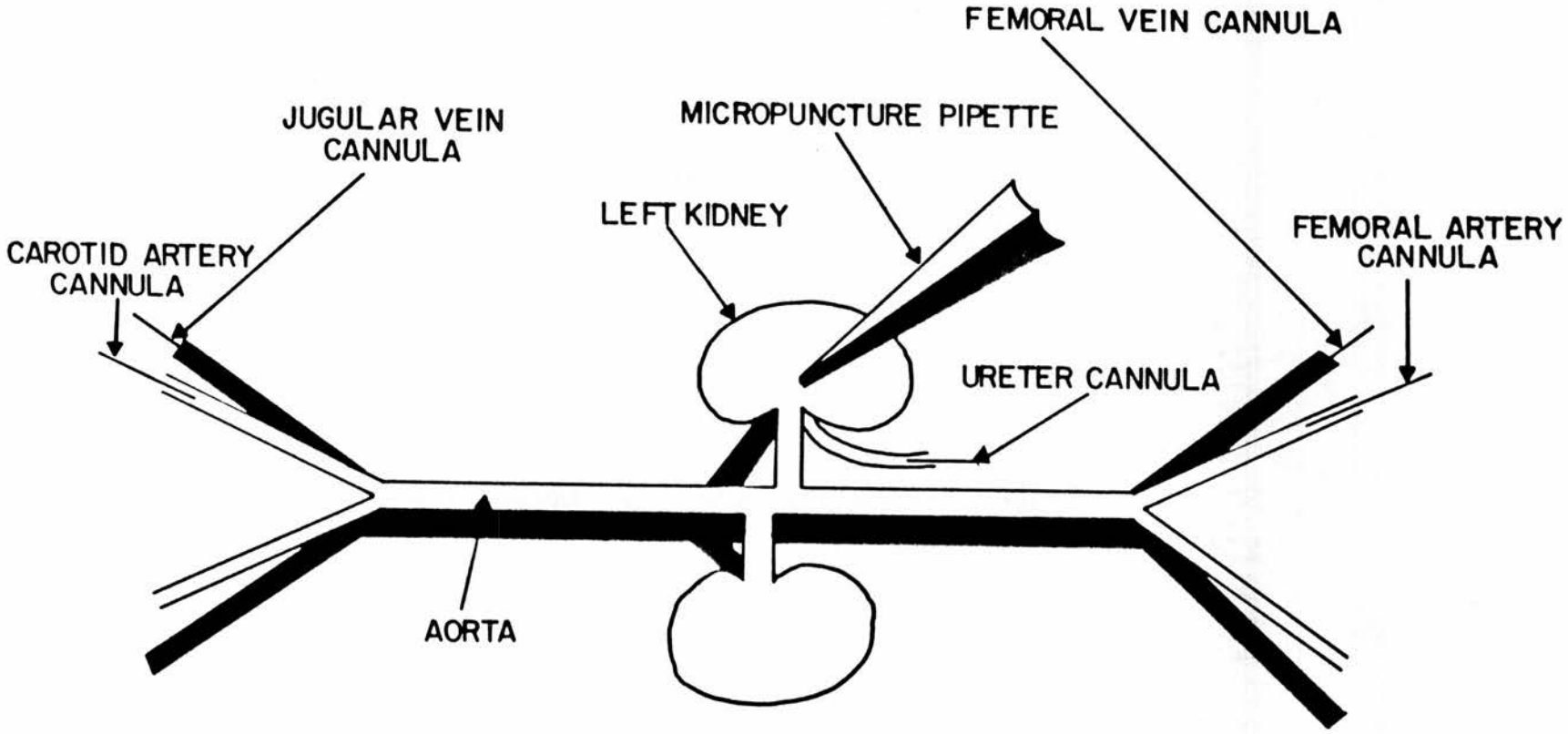


FIG 2 SCHEMATIC REPRESENTATION OF THE POSITIONS OF CANNULAE IN ANAESTHETISED RATS

Thus, the experiments reported in Section 5 were designed to evaluate the effects of systemic infusion of low doses of AII on renal function, in particular proximal tubular fluid reabsorption, in chronically sodium loaded rats. The rats were chronically sodium loaded in an attempt to suppress endogenous angiotensin production and thereby permit the infused hormone to elicit a pronounced antidiuresis and antinatriuresis. Two groups of rats were used. In one group, AII was infused at a rate of 0.1 $ng.min^{-1}$ for one hour, while in the other group AII was infused at successive rates of 1 and 5 $ng.min^{-1}$ each for one hour.

Infusion of AII at rates of 0.1, 1 and 5 $ng.min^{-1}$ were found not to significantly affect renal excretory function in the experiments reported in Section 5. These findings were interpreted as indicating that in the chronically sodium loaded rat, endogenous angiotensin production is not suppressed and that AII is still produced in amounts that are optimal for maximal sodium retention. Thus, in the experiments reported in Section 6, alternative methods of suppressing endogenous angiotensin production were employed in an attempt to facilitate the demonstration of a pronounced antinatriuretic effect of infused AII. Two groups of rats were used. In one group, rats were chronically sodium loaded and acutely volume expanded in an attempt to physiologically suppress the activity of the renin-angiotensin system. AII was infused at successive rates of 5 and 10 $ng.min^{-1}$ each for one hour. In the second group, the rats were again chronically sodium loaded and acutely volume expanded; in addition, these rats were treated with the angiotensin converting enzyme inhibitor (CEI), captopril, to pharmacologically suppress the activity of the renin-angiotensin system. AII was infused at a rate of 10 $ng.min^{-1}$ for one hour during continuing converting enzyme inhibition.

The rationale and experimental procedures for each group of rats are described in more detail in Sections 3-6.

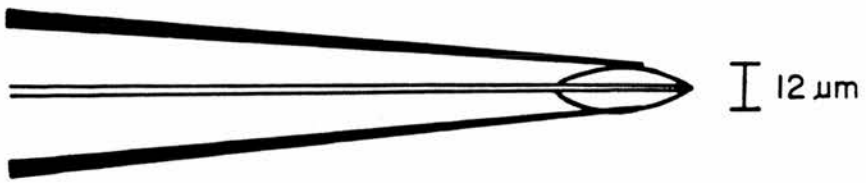
MICROPIPETTES

Double barrelled glass micropipettes were made from lengths of borosilicate capillary tubing containing a glass fibre filament (Theta glass, Cat. No. TGC 150-15, Clark Electromedical Instruments, England). Before pulling the micropipettes, the capillary tubing was cleaned by soaking overnight in chromic acid. The tubing was then rinsed repeatedly with tap and distilled water and put in an oven to dry. Micropipettes with tip diameters ranging between 1 and 15 μm were pulled using a Livingston pipette puller (LPP-2, Clark Electromedical). The tip of each micropipette was viewed under a Stereomicroscope (Olympus VMF-4) and cut to a diameter of 12-15 μm using fine watchmaker forceps. The tip of each micropipette was then bevelled to an angle of 45° on the diamond pasted wheel of a motor driven grinding machine (Wolfgang Hampel, West Germany). After grinding, the micropipette tips were cleaned by repeated immersions in chromic acid and distilled water. To keep the tips dust free and to protect them from damage, the pipettes were stored in an inverted glass beaker until used. Fig. 3 shows a diagram of the tip of a double barrelled glass micropipette manufactured as described above.

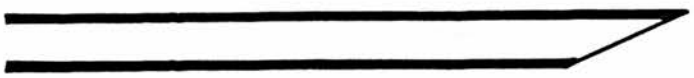
SHRINKING SPLIT OIL DROPLET TECHNIQUE

The method described by Gertz (1963) and modified by Gyory (1971) was used to determine the rate of tubular fluid reabsorption (Jv) in straight segments of surface proximal tubules. Briefly, a proximal tubule was punctured with a double-barrelled glass micropipette (see Fig. 3), one barrel of which was filled with Sudan black stained castor oil and the other with an aqueous test solution (intratubular perfusion fluid). The intratubular perfusion fluid always contained Na^{+} (150 mmol.kg^{-1}), Cl^{-} (135 mmol.kg^{-1}), Ca^{++} (1.5 mmol.kg^{-1}), Acetate (10 mmol.

FIG 3 THE TIP OF A DOUBLE BARRELLED GLASS MICROPIPETTE



FRONT VIEW



SIDE VIEW

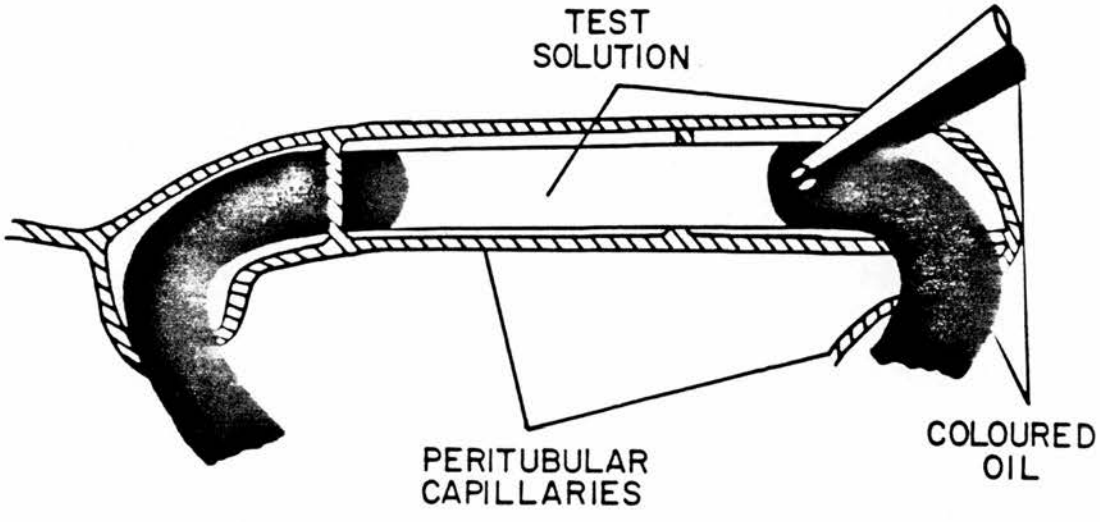
kg^{-1}) and HCO_3^- (8 mmol.kg^{-1}) and had a real osmolality of 283 mosm. $kg^{-1}H_2O$. The ionic composition and osmolality of the intratubular perfusion fluid were chosen so as to be similar to that of mid-proximal luminal fluid. The intratubular perfusion fluid was made using only Analar grade chemicals and fresh distilled water. It was stored at 4°C and was kept for a maximum of 5 days. Prior to each experiment, the intratubular perfusion fluid was filtered through a millipore filter (3 μm pore size).

A long column of castor oil (about 30-50 tubular diameters) was injected into the tubule and then a small volume of intratubular fluid was injected so as to split the oil column into two. The droplet was then moved upstream from the pipette by injecting a little more oil. An isolated droplet is shown diagrammatically in Fig. 4. The isolated droplet was kept upstream from the site of puncture by maintaining a slight pressure on the distal oil column. Droplets were photographed on Ilford black and white photographic film using a 16 mm cine camera (Vinten) triggered to expose one frame per second. A fibre optic light source (Volpi) was used to illuminate the kidney surface for micropuncture and photography. In order to ensure that the rate of fluid reabsorption was measured at steady state luminal pressure, the first picture of each sequence was taken at least five seconds after the droplet was formed.

DEVELOPMENT OF CINE FILM

The negatives on the 16 mm cine film were developed at 20°C using commercial black and white film developer (Ilford Microphen), and fixed using Kodafix solution (Kodak, England). All stages of the film development were carried out in a dark room.

FIG 4 DIAGRAM OF AN ISOLATED DROPLET POSITIONED IN A SURFACE SEGMENT OF A PROXIMAL TUBULE



INULIN ASSAY METHODS

Either polyfructosan (Inutest, Laevosan Gessellschaft, Linz, Austria) or ^{14}C inulin (Inulin (^{14}C) Carboxylic acid, specific activity 3.2 $mCi.mmol^{-1}$, Amersham International PLC, Buckinghamshire, England) was used as the clearance marker for the estimation of glomerular filtration rate (GFR). Thus, depending on which clearance marker was used, one of the following methods was employed to determine the inulin concentration in urine and plasma.

(a) ANTHRONE METHOD

Urine and plasma polyfructosan concentrations were determined using the modification by Brenner (see Andreucci, 1978b) of the method initially described by Young and Raisz (1952). Briefly, aliquots (250 µl) of each sample (deproteinised plasma, urine, Inutest standard or distilled water) were added to tubes containing 2.5 ml Anthrone reagent, mixed and heated at 55°C in a water bath for ten minutes. The samples were cooled rapidly, poured into cuvettes and the absorbance at 530 nm determined (Unicam SP500 Series 2 Spectrophotometer). Protein was precipitated from plasma samples by adding aliquots (50 µl) to tubes containing distilled water (750 µl) and TCA reagent (200 µl). The tubes were centrifuged for 15 minutes and the supernatant removed. Urine and plasma inulin concentrations were determined in duplicate using a standard curve constructed using Inutest standards (0.01 - 0.1 $mg.ml^{-1}$). A blank for the standard curve was constructed by adding 250 µl distilled water to 2.5 ml Anthrone reagent. The standard colorimetric curve was nearly linear and quite reproducible.

(b) LIQUID SCINTILLATION COUNTING TECHNIQUE

Urine and plasma ^{14}C inulin concentrations were measured in dupli-

cate in an automatic scintillation counter (KB-Wallac (1218) Rackbeta). Aliquots (20 µl) of urine and plasma samples were added to polyethylene vials containing 3 ml Rialuma scintillation fluid (Lumac, Holland). The vials were sealed, shaken vigorously and loaded into the scintillation counter. All samples had the same degree of quenching and an activity at least ten times that of background. The scintillation counter had a high degree of accuracy and reproducibility.

Glomerular filtration rate (GFR) was calculated using the standard clearance formula, and expressed per gram kidney weight.

GFR ($ml.min^{-1}.g^{-1}$) = (U/P inulin ratio x urine flow rate)/kidney weight.

SODIUM AND POTASSIUM DETERMINATIONS

Sodium and potassium concentrations in plasma and urine were determined using a Corning 455 flame photometer (Corning, England). Sample dilutions were made where appropriate with distilled water.

ANALYSIS OF CINE PHOTOGRAPHS

The images of shrinking droplets on the black and white negatives were projected onto a white screen using a single frame analysis projector (Model 244 A Special II; L-W International, Calif, U.S.A.) and the shrinking droplet sequences were analysed by hand using a dial caliper (Mitutoyo, Japan). Analysis of split droplets by hand was performed by the same person and standardised as much as possible in order to minimise potential sources of analytical error.

Shrinking droplet half-times ($t_{\frac{1}{2}}$) were calculated from the semi-logarithmic relationship between the corrected droplet length and time (Gyory, 1971). Since comparisons of tubular reabsorptive capacity were to be made between tubules within each rat and between different rats, the rate of reabsorption was expressed as flux per unit surface area [Jv(a)] (Gyory, 1971). All calculations of $t_{\frac{1}{2}}$ and Jv(a) were

performed on a Cromemco Z-2D system two computer.

In the time control experiments (group 3) reported in Section 4, estimates of Jv were obtained from four or five tubules in each of the five rats during the first 60 minute infusion period (only one estimate of Jv per tubule). For the five sets of Jv measurements, the mean coefficient of variation was 22.3%. This value is slightly higher than the 17% reported by Gyory (1971). Since comparisions of tubular reabsorptive capacity were to be made between tubules within each rat and between different rats, the reproducibility of repetitive measurements of Jv within the same tubular segment was not determined.

CALCULATIONS AND STATISTICAL ANALYSIS

The rate of urine production was determined gravimetrically. Arterial haematocrit was measured using heparinised microhaematocrit tubes (Hawksley, Ltd.). Mean arterial blood pressure (MABP) was calculated as diastolic + 1/3 pulse pressure. Values for MABP were recorded at 15 minute intervals during each experiment and were averaged for each 60 minute period for each animal. Estimates of Jv were obtained from one to six tubules in each animal during each 60 minute infusion period (only one estimate of Jv per tubule). Jv values and excretory variables were averaged for each 60 minute infusion period in each animal. All results refer to the overall mean values determined for each 60 minute infusion period.

Results are expressed as means ± SEM. A one-way analysis of variance was used to determine whether differences existed between control period values of different groups. A two-way analysis of variance was used to evaluate changes in paired studies (ie, between control and experimental periods). Split plot two-way analysis of variance with repeated measures was used to evaluate changes among groups. Significance was tested using Duncan's multiple range test set at the $P<0.05$

level.

CHEMICALS AND DRUGS

All chemicals used were Analar grade. Angiotensin I (ile^5-acetate: Sigma); angiotensin II (val^5-amide; Ciba-Geigy); captopril (SQ 14,225; Squibb). All solutions were made up in 0.9% sterile saline at a stock concentration of 10^{-5}M and stored in siliconised sterile ampoules at -20°C. Final dilutions were made in plastic tubes during the experiment.

SECTION 3

PRESSURE DEPENDENCE OF ANGIOTENSIN-INDUCED NATRIURESIS IN RATS

INTRODUCTION

Angiotensin II has been demonstrated to exert a dose-dependent effect on urinary sodium and water excretion in man, rabbit, dog, monkey and rat (Navar and Langford, 1974). Infusion of low or sub-pressor doses of AII (1-50 $ng.kg^{-1}.min^{-1}$) produces a decrease in urine flow and sodium excretion rate (Barraclough, 1965; Barraclough et al., 1967; Malvin and Vander, 1967; Johnson and Malvin, 1977). Infusion of high or pressor doses of the peptide elicit a biphasic effect on urine flow and sodium excretion rate, ie, there is an initial decrease followed by a marked increase.

The initial phase of the response to pressor doses of AII may be explained, as can the response to low doses, by the concomitant decreases in RBF and GFR. Although the mechanism responsible for the natriuretic phase of the response is still not clear, several groups have reported that the natriuretic response is not related to the pressor response (Villarreal, et al., 1964; Louis and Doyle, 1965; Healy and Elliott, 1971). These results suggest that a direct inhibitory effect of the peptide on tubular reabsorption may be responsible for the natriuretic response.

However, no micropuncture study has been carried out in order to determine whether a direct inhibitory effect of AII on tubular reabsorption is responsible for the observed natriuresis. Thus, the experiments described in this section were carried out to determine the renal effects of a pressor dose of AII and to determine the mechanism of angiotensin induced natriuresis in anaesthetised rats.

METHODS

ANIMALS

The experiments were performed on male Wistar rats (250-300g) allowed free access to standard rat diet and tap water.

SURGICAL PROCEDURE

The rats were anaesthetised and surgically prepared for micropuncture as described in Section 2. However, only one cannula was inserted into the left jugular vein. This cannula was attached to a syringe containing 0.9% NaCl and operated by a constant infusion pump (Braun) set to deliver 75 $\mu l.min^{-1}$. In addition to a left carotid artery cannula, a cannula was inserted into the left femoral artery to allow continuous monitoring of left femoral arterial pressure. Also, a polyethylene snare was placed around the aorta between the origins of the left and right renal arteries.

EXPERIMENTAL PROCEDURE

Immediately after placement of the jugular cannula, a priming injection of 1 ml of 0.9% NaCl was given to replace fluid losses during surgery and 0.9% NaCl was infused at 75 $\mu l.min^{-1}$. Upon completion of surgery, a period of 60 minutes was allowed for stabilisation of urine production and electrolyte excretion.

The equilibration period was followed by a control period of 30-60 minutes during which 0.9% NaCl only was infused. Angiotensin II was then added to the infusate to give a delivery rate equivalent to 100 $ng.min^{-1}$ ($10^{-10}M.min^{-1}$) and infused for 60 minutes. There then followed a recovery period of saline infusion. Urine was collected over 10-20 minute periods throughout the experiments.

Two groups of animals were used.

Group 1 - Control group (n=7)

The increase in MABP that occurred during the AII infusion period was allowed to increase the perfusion pressure of the left kidney.

Group 2 - Clamped group (n=7)

The left femoral artery cannula monitored arterial pressure distal to the aortic snare and thus the arterial perfusion pressure of the left kidney. During the AII infusion period, the snare was tightened to prevent a rise in arterial pressure from increasing the perfusion pressure of the left kidney. The snare was released at the end of the AII infusion period.

Since the experimental procedure for both groups 1 and 2 included a recovery period, it was not considered necessary to perform separate time control experiments.

Throughout the experiments, randomly selected proximal tubules were micropunctured and rates of fluid reabsorption were determined using the shrinking droplet micropuncture technique (Gyory, 1971). The micropuncture techniques are described in Section 2. At the end of each experiment, the left kidney was removed, blotted dry and weighed.

RESULTS

In both groups 1 and 2, urine flow and urinary sodium excretion did not vary significantly during the initial control period, suggesting that steady state conditions had been attained.

MEAN ARTERIAL BLOOD PRESSURE (MABP)

Mean values for the left carotid (group 1) and left femoral (group 2) arterial blood pressure are shown in Fig. 5. Mean arterial blood pressure during the control period in group 1 was not significantly different from that in group 2. In group 1, MABP increased from 120.7± 3.4 mmHg to 149.6 ± 4.3 mmHg ($P<0.05$) during AII infusion. It was noticeable that the increase in blood pressure was present throughout the duration of the AII infusion. This differs from reports that blood pressure falls towards control levels during infusion of a pressor dose of angiotensin (Villarreal et al., 1964; Louis and Doyle, 1965). During the recovery period, arterial blood pressure decreased to 112.0 ± 3.7 mmHg, a value significantly lower than during the control period ($P<0.05$).

There was no significant change in left femoral arterial blood pressure during the experiments in group 2. Thus, left kidney perfusion pressure (as indicated by left femoral arterial pressure) was kept constant throughout the AII infusion period.

URINE FLOW (Fig. 6)

In the control group (group 1), urine flow was 13.02 ± 2.63 $\mu l.min^{-1}$ during the control period and 8.33 ± 2.00 $\mu l.min^{-1}$ during the first 20 minutes of AII infusion. During the latter 40 minutes of infusion, urine flow increased to 18.56 ± 3.27 $\mu l.min^{-1}$, a level significantly

FIG 5 THE EFFECT OF ANGIOTENSIN II ON MEAN ARTERIAL BLOOD PRESSURE

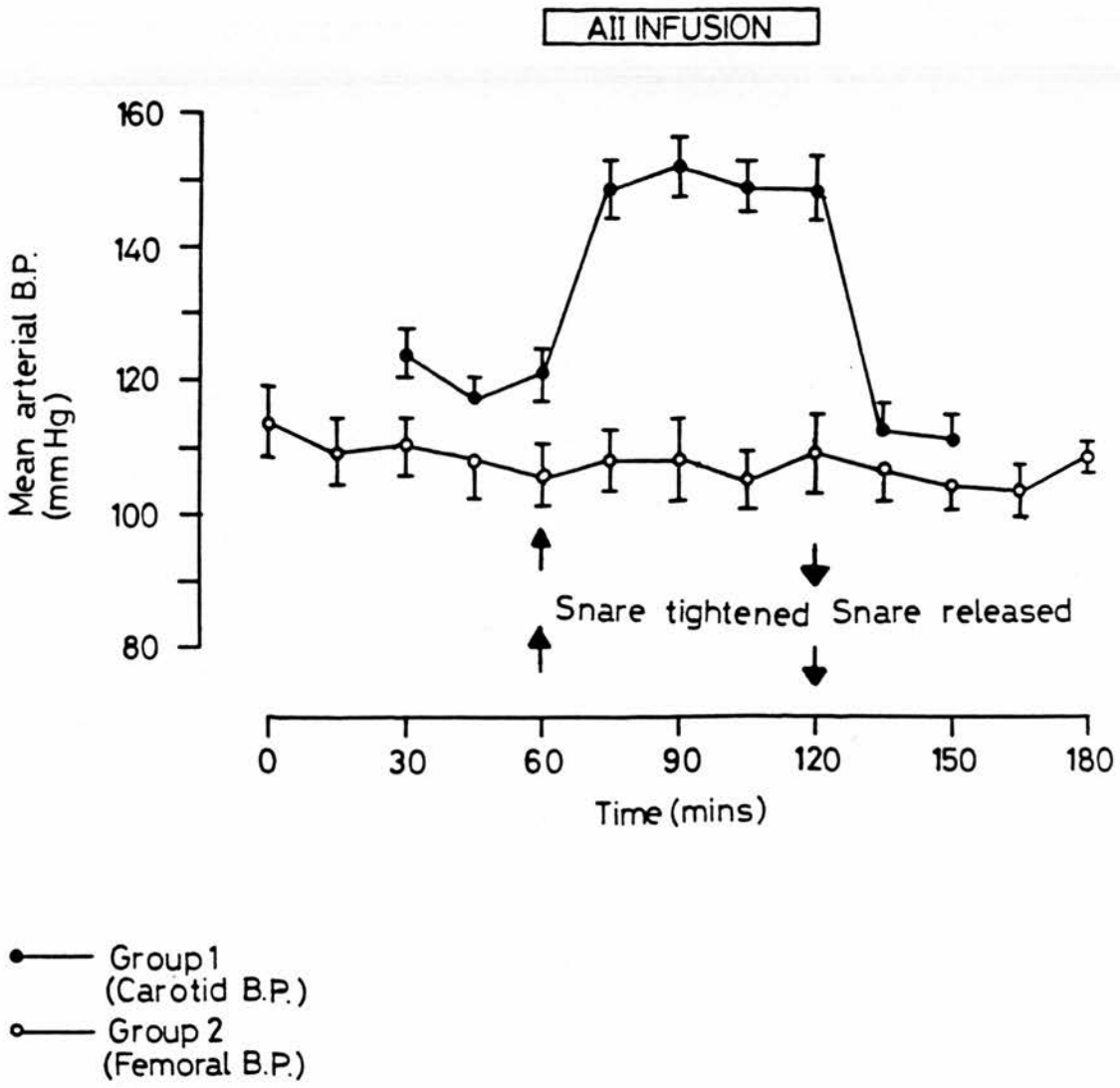
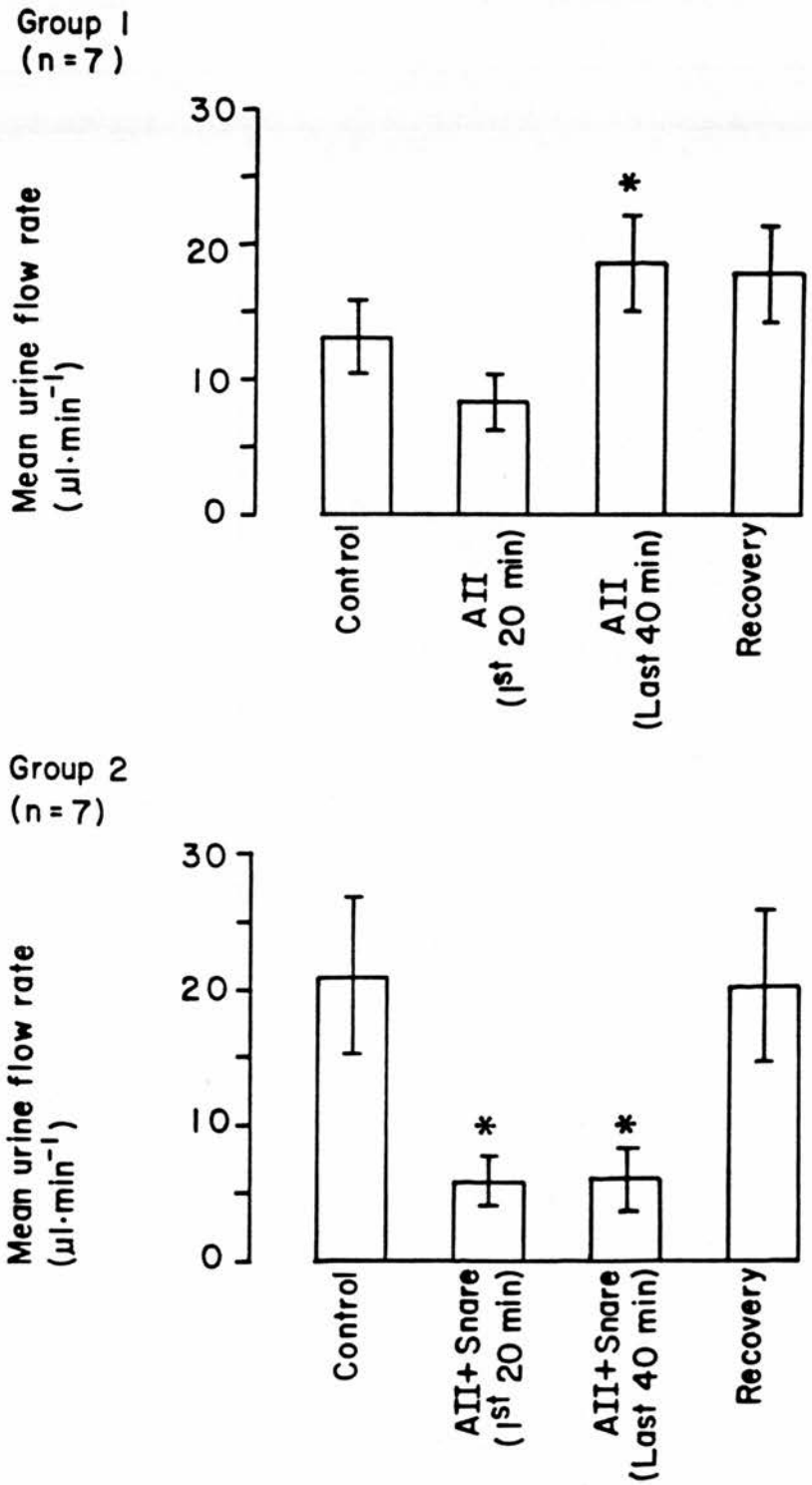


FIG 6 THE EFFECT OF ANGIOTENSIN II ON MEAN URINE FLOW RATE



*, P<0.05 relative to control

higher than that during the control period ($p<0.05$). During the recovery period, urine flow was 17.84 ± 3.58 $\mu l.min^{-1}$, a value not significantly different from the control value.

In the clamped group (group 2), urine flow decreased significantly from 20.93 ± 5.9 $\mu l.min^{-1}$ during the control period to 5.90 ± 1.88 ($P<0.05$) during the first 20 minutes of AII infusion and to 6.22 ± 2.52 $\mu l.min^{-1}$ ($P<0.05$) during the last 40 minutes of AII infusion. During the recovery period, urine flow increased to 20.22 ± 5.54 $\mu l.min^{-1}$, a value not significantly different from the control value (Fig. 6).

SODIUM EXCRETION RATE

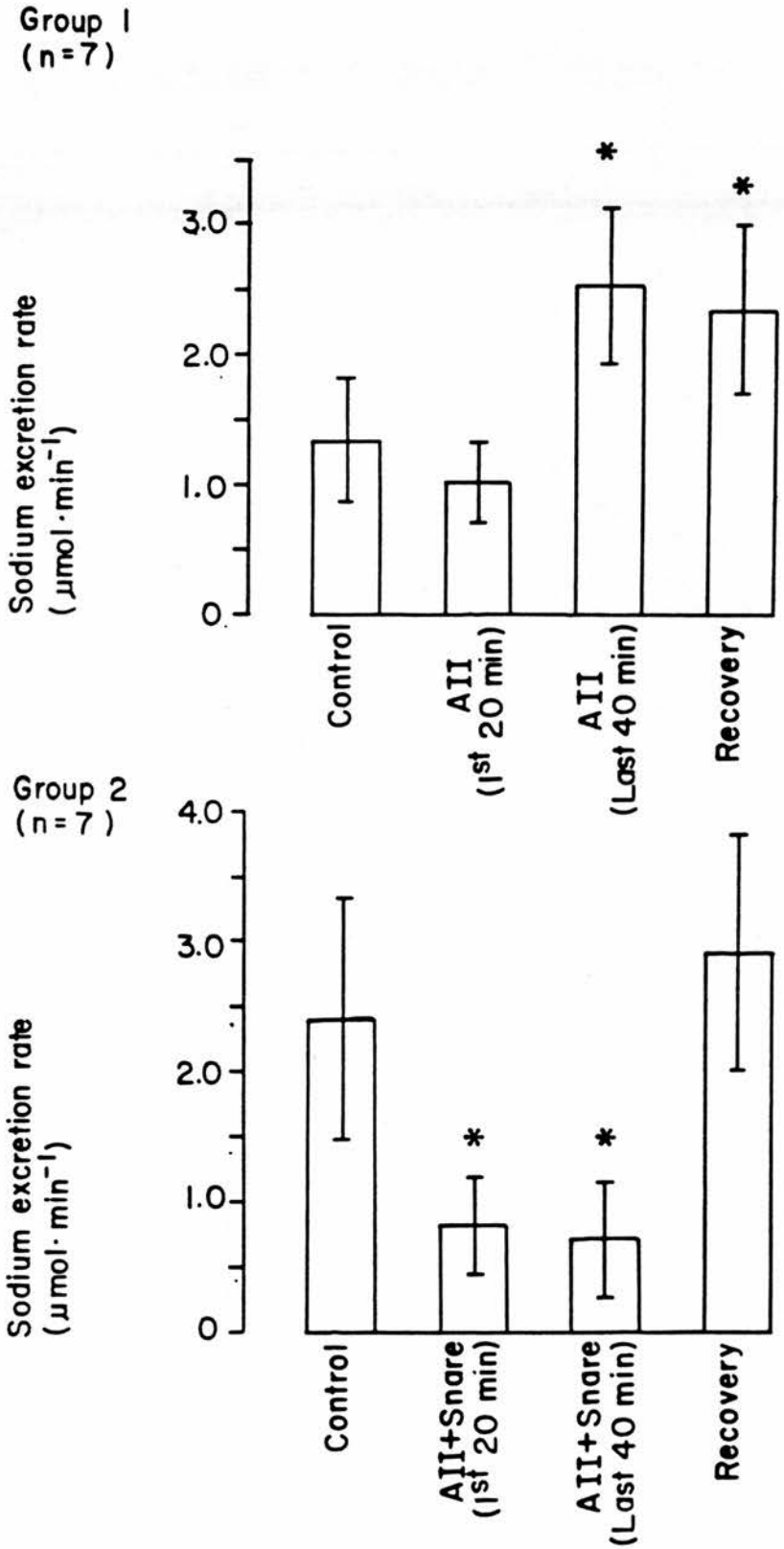
Data obtained for sodium excretion rates are shown in Fig. 7. In the control group (group 1), sodium excretion rate was 1.02 ± 0.32 $\mu mol.min^{-1}$ during the first 20 minutes of AII infusion, a value not significantly different from the control value (1.34 ± 0.48 $\mu mol.min^{-1}$). During the last 40 minutes of AII infusion, the sodium excretion rate increased to 2.54 ± 0.60 $\mu mol.min^{-1}$, a value significantly higher than during the control period ($P<0.05$). Sodium excretion remained elevated at 2.35 ± 0.65 $\mu mol.min^{-1}$ during the 30 minute recovery period ($P<0.05$).

In the clamped group (group 2), sodium excretion decreased from 2.40 ± 0.94 $\mu mol.min^{-1}$ during the control period to 0.82 ± 0.37 ($P<0.05$) during the first 20 minutes of AII infusion, and to 0.72 ± 0.44 $\mu mol.min^{-1}$ ($P<0.05$) during the last 40 minutes of AII infusion. During the recovery period, the sodium excretion rate increased to 2.92 ± 0.91 $\mu mol.min^{-1}$, a value similar to the control value.

POTASSIUM EXCRETION RATE

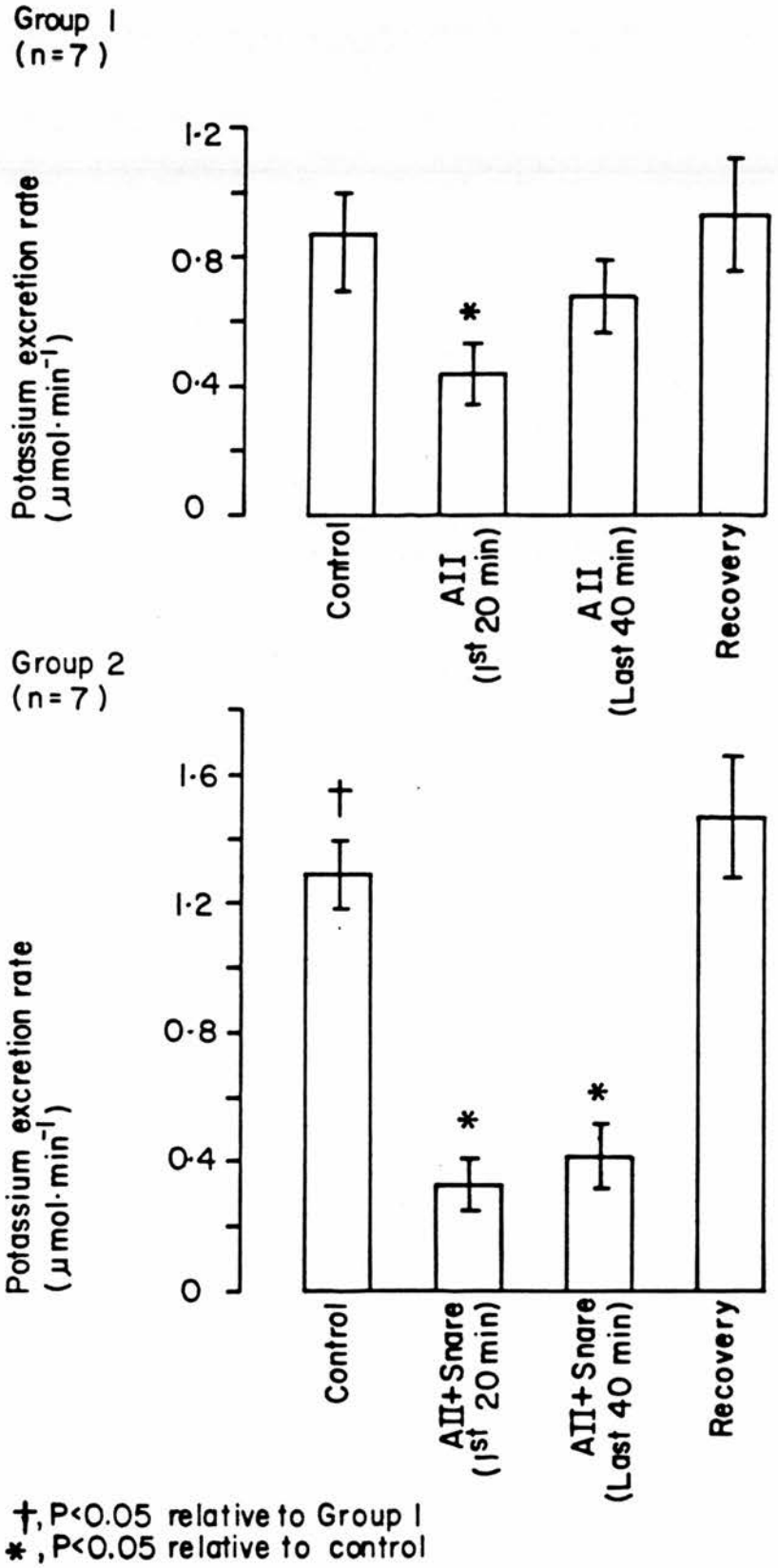
Mean potassium excretion during the control period in group 1 was 0.87 ± 0.18 $\mu mol.min^{-1}$, a value significantly lower ($P<0.05$) than the

FIG 7 THE EFFECTS OF ANGIOTENSIN II ON MEAN SODIUM EXCRETION RATE



*, P<0.05 relative to control

FIG 8 THE EFFECTS OF ANGIOTENSIN II ON MEAN POTASSIUM EXCRETION RATE



†, P<0.05 relative to Group I
*, P<0.05 relative to control

control value in group 2 (1.29 ± 0.11 μmol.min^{-1}). The reason for this difference is unclear.

In group 1, the potassium excretion rate decreased from 0.87 ± 0.18 μmol.min^{-1} during the control period to 0.44 ± 0.10 μmol.min^{-1} during the first 20 minutes of AII infusion ($P<0.05$). During the latter 40 minutes of AII infusion, potassium excretion rate increased to 0.68 ± 0.11 μmol.min^{-1}, and although this remained below the control value, the difference was not statistically significant. During the recovery period, the potassium excretion rate was 0.93 ± 0.17 μmol.min^{-1}, a value not significantly different from the control value (Fig. 8).

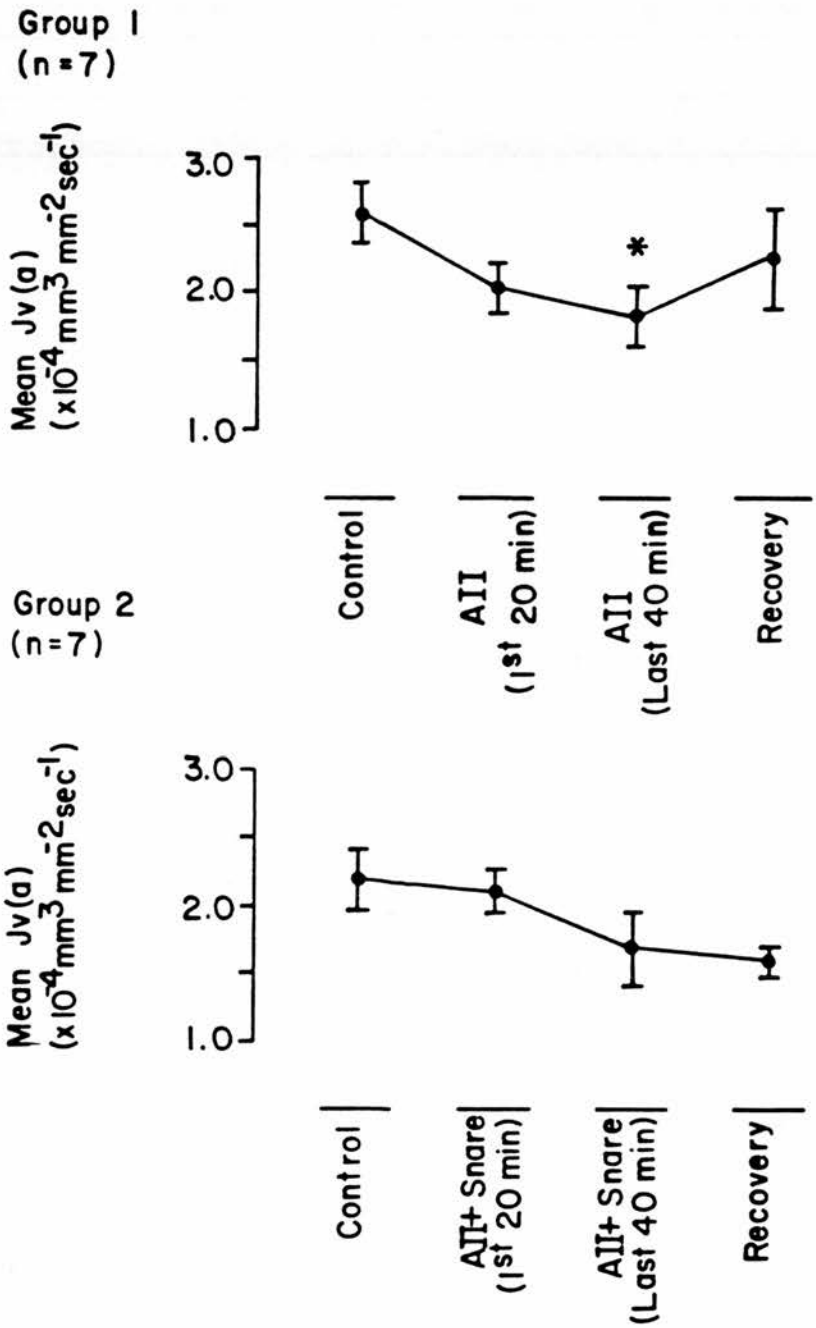
In the clamped group (group 2), the potassium excretion rate decreased from 1.29 ± 0.11 μmol.min^{-1} during the control period to 0.33 ± 0.08 ($P<0.05$) during the first 20 minutes of AII infusion and to 0.42 ± 0.10 μmol.min^{-1} ($P<0.05$) during the last 40 minutes of AII infusion. During the recovery period, potassium excretion rate increased to 1.47 ± 0.19 μmol.min^{-1}, a value not significantly different from the control value (Fig. 8).

PROXIMAL TUBULAR FLUID REABSORPTION (Jv)

Data obtained for mean Jv during the control, first 20 minute phase of AII infusion, the last 40 minute phase of AII infusion and the recovery period are shown in Fig. 9. Mean Jv during the control period in the clamped group (group 2) was 2.19 ± 0.23 x 10^{-4} mm^3.mm^{-2}.sec^{-1}, a value not significantly different from the corresponding value in the control group (group 1). As shown in Fig. 9, mean Jv did not vary significantly during the course of the experiment in group 2.

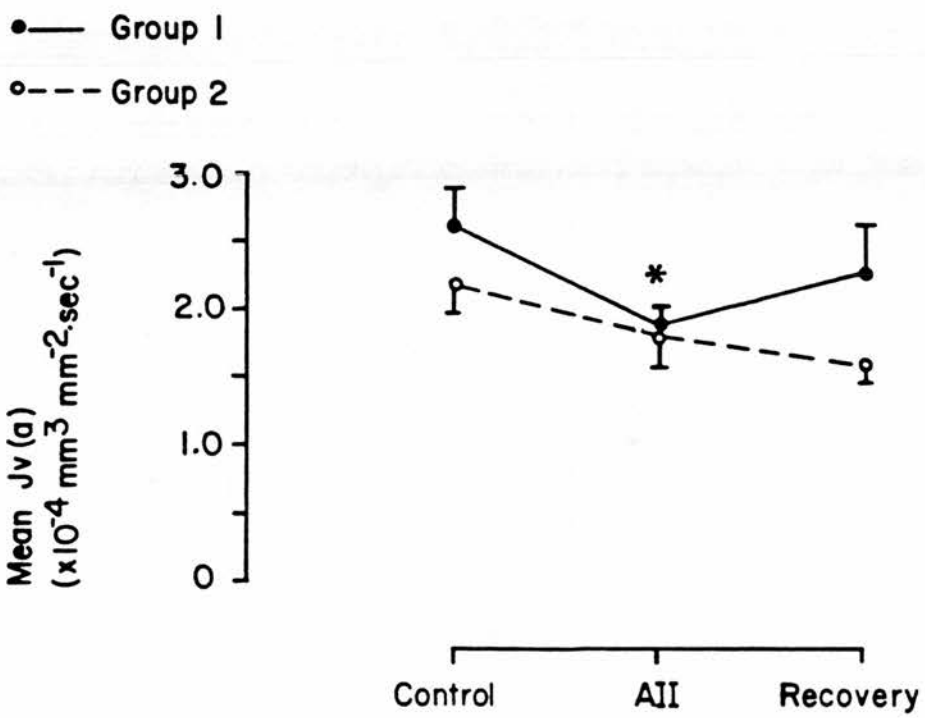
In the control group (group 1), mean Jv was 2.61 ± 0.23 during the control period and 2.05 ± 0.20 during the first 20 minutes of AII infusion, but decreased to 1.83 ± 0.22 x 10^{-4}mm^3.mm^{-2}.sec^{-1} during the last 40 minutes of AII infusion ($P<0.05$). During the recovery period,

FIG 9 THE EFFECT OF ANGIOTENSIN II ON PROXIMAL TUBULAR FLUID REABSORPTION (Jv(a))



*, $P < 0.05$ relative to control

FIG 10 THE EFFECTS OF ANGIOTENSIN II ON MEAN PROXIMAL TUBULAR FLUID REABSORPTION (Jv(a))



*, P<0.05 relative to control

mean Jv was 2.26 ± 0.37 x 10^{-4} $mm^3.mm^{-2}.sec^{-1}$, a value not significantly different from that observed during the control period, or the last 40 minutes of AII infusion.

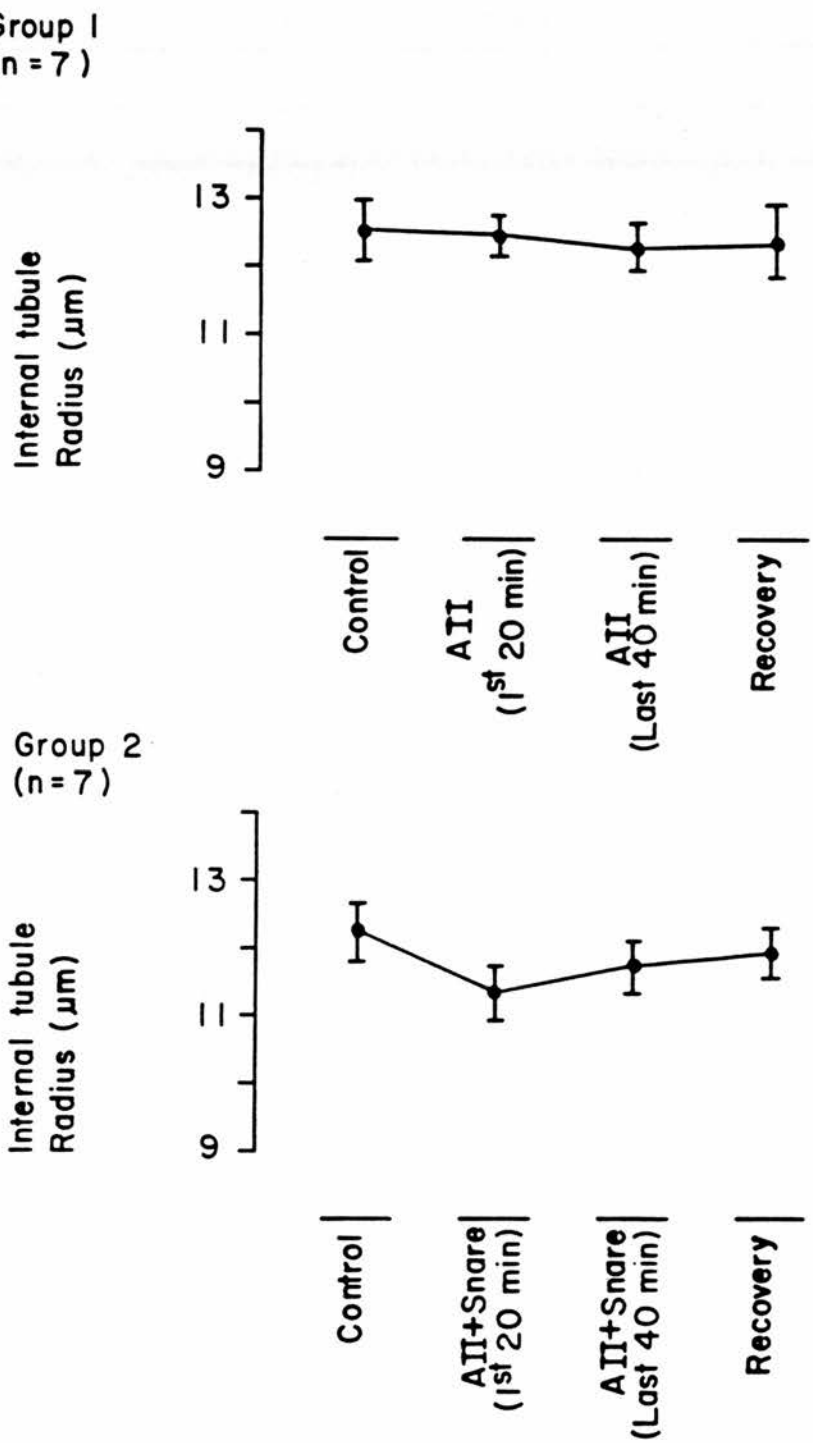
Figure 10 shows the changes in mean Jv in the two groups of rats when overall Jv for each AII infusion period was calculated. In the control group (group 1), mean Jv was 1.87 ± 0.15 x 10^{-4} $mm^3.mm^{-2}.sec^{-1}$ during the AII infusion period, a value significantly lower than the control value (2.61 ± 0.28 x 10^{-4} $mm^3.mm^{-2}.sec^{-1}$, $P<0.05$) but not significantly different from the recovery period value (2.26 ± 0.37 x 10^{-4} $mm^3.mm^{-2}.sec^{-1}$). In the clamped group (group 2) mean Jv did not vary significantly during the course of the experiment.

INTERNAL TUBULE RADIUS

As shown in Fig. 11, mean internal tubule radius did not vary significantly in either group.

FIG II

THE EFFECT OF ANGIOTENSIN II ON INTERNAL TUBULE RADIUS



DISCUSSION

The results demonstrated that infusion of AII at a rate of 100 $ng.min^{-1}$ resulted in a significant increase in arterial blood pressure. This increase was maintained throughout the duration of the AII infusion (group 1, Fig. 5). During the first 20 minutes of AII infusion both urine flow and urinary sodium excretion were not different from the initial control values, although the potassium excretion rate was found to be significantly reduced. However, both urine flow and sodium excretion increased significantly during the latter 40 minutes of AII infusion. During the recovery period, urine flow and sodium excretion were not significantly different from that observed during the latter 40 minutes of AII infusion. Indeed, sodium excretion during the recovery period was significantly higher than during the initial control period (group 2, Fig. 7). In addition, the rate of proximal tubular fluid reabsorption during the latter 40 minutes of AII infusion was lower than during the control period but not significantly different from that during the recovery period. Thus, it is possible that recovery of urine flow, sodium excretion and proximal reabsorption to control levels following infusion of a natriuretic dose of AII may take longer than 30 minutes, and that in this respect, a longer recovery period may have been more appropriate.

Although the mechanism responsible for the natriuretic response is unclear, it has been proposed that it is due to a direct inhibitory effect of the hormone on tubular reabsorption independent of the associated increase in arterial blood pressure (Healy and Elliott, 1971; Navar and Langford, 1974). However, the results obtained from the second group of animals do not support this hypothesis. Indeed, when the perfusion pressure to the left kidney was prevented from increasing during the AII infusion, there was a significant reduction in both urine

flow and sodium excretion rate. These changes occurred in the absence of a significant alteration in the rate of proximal tubular fluid reabsorption (group 2, Fig. 10).

These results suggest that in the anaesthetised rat, the natriuretic response to AII is dependent upon the increase in arterial blood pressure and does not involve a direct inhibitory effect of high doses of AII on proximal tubular reabsorption. These findings are in contrast to those of Healy and Elliott (1971) who demonstrated that angiotensin induced natriuresis in rabbits occurred even when the increase in blood pressure was prevented by haemorrhage. These differences may be due to a species difference, an effect of the anaesthetic, or the use of different procedures to control renal perfusion pressure during the infusion of a pressor dose of angiotensin II.

In the present study, infusion of AII resulted in a decrease in proximal Jv only when the accompanying rise in arterial blood pressure was allowed to increase renal perfusion pressure. This indicates that the observed decrease in proximal Jv was due to the increase in perfusion pressure per se and not due to a direct inhibitory effect of the peptide on proximal tubular fluid reabsorption. It has been suggested that an increase in renal perfusion pressure results in an increase in peritubular capillary and interstitial hydrostatic pressure (Ott et al., 1971; Earley and Schrier, 1973; Knox et al., 1983). In addition, using the shrinking droplet micropuncture technique, Sato (1975) demonstrated that increases in peritubular capillary hydrostatic pressure decreased proximal tubular fluid reabsorption. Thus, it is possible that the natriuretic response to a pressor dose of AII may be due, in part, to a reduction in proximal tubular fluid reabsorption, caused by increased capillary and/or interstital hydrostatic pressure secondary to the increased renal perfusion pressure.

The finding that internal tubule radius did not vary significantly in group 1 suggests that the reduction in proximal Jv was not due to an alteration in tubular geometry secondary to the increased perfusion pressure. Similarly, internal tubule radius did not vary significantly in group 2, indicating that there was no direct effect of AII on tubular geometry.

Several studies have provided evidence that the loop of Henle is a major site at which alterations in intrarenal physical factors affect urinary sodium and water excretion. Indeed, it was shown that in dogs, an increase in renal arterial pressure resulted in increases in both sodium excretion and free water clearance (Selkurt et al., 1965). Bank et al., (1970) reported that elevation of systemic arterial blood pressure by infusion of adrenaline, produced a decrease in sodium reabsorption by the loop of Henle and an increase in sodium excretion rate. Therefore the natriuretic response to a pressor dose of AII, may also be due in part, to reduced reabsorption by the loop of Henle, caused by increased capillary and/or interstitial hydrostatic pressure secondary to the increased renal perfusion pressure. An increase in capillary and/or interstitial hydrostatic pressure may decrease the hydrostatic pressure gradient across the wall of the thin descending limb and thus decrease passive movement of water out of this segment. This would result in a larger volume of less concentrated fluid being delivered to the ascending limb and the distal tubule, and thus a natriuresis. However, an alternative possibility is that the natriuretic response to AII may be consequent on an increased glomerular filtration rate secondary to the increased renal perfusion pressure. As GFR was not measured in the present study, it is not possible to determine whether or not the changes in urinary excretion were a consequence of an altered GFR.

In the present study, arterial blood pressure and thus renal perfusion pressure increased immediately following administration of AII in group 1. However, both urine flow and urinary sodium excretion were not significantly altered during the first 20 minutes of AII infusion. It is possible that during this period, AII may have produced a marked intrarenal vasoconstriction thereby offsetting the diuretic and natriuretic effect of the elevated perfusion pressure. The increase in urine flow and urinary sodium excretion that occurred during the last 40 minutes of AII infusion may have been due to a waning of the intrarenal vasoconstriction thereby allowing the elevated perfusion pressure to elicit a diuresis and natriuresis. The observation that when renal perfusion pressure was prevented from increasing, infusion of AII produced a sustained antidiuresis and antinatriuresis could be interpreted as support for the possibility that the waning of the intrarenal vasoconstrictor effect of AII is dependent upon an increase in renal perfusion pressure.

It is questionable whether endogenous angiotensin production ever reaches such natriuretic levels. Since a viable AII assay was not available, AII levels in the plasma or kidney tissue were not measured in this study. Therefore, it is not known what the endogenous AII levels were, or to what levels the circulating and/or tissue concentrations were raised during infusion of the peptide. However, Brown et al., (1981) measured plasma AII concentrations in conscious rats receiving systemic infusions of various doses of the peptide. They reported that infusion of AII at the rate of 270 $ng.kg.^{-1}min^{-1}$ increased circulating levels to approximately 3 ng/ml ($3 \times 10^{-9}M$; a thirty-two fold increase compared with controls infused with dextran). In the present study, AII was infused at a rate of 100 $ng.min^{-1}$; equivalent to 333-400 $ng.kg.^{-1}\ min^{-1}$. Despite the fact that anaesthetised rats were used in this study, it would be reasonable to assume that the doses of

AII employed in the present study resulted in a circulatory concentration in excess of 10^{-9}M. Although it is unlikely that such high concentrations occur in the normal control of sodium excretion, they might occur in pathological conditions. Indeed, very high circulating levels of the peptide (in excess of 10^{-9}M) have been reported in the rat under conditions of sodium restriction (Oster et al., 1973) and haemorrhagic hypotension (Semple, 1981).

SECTION 4

THE EFFECT OF INFUSION OF SUB-PRESSOR DOSES OF ANGIOTENSIN II ON RENAL FUNCTION IN RATS

INTRODUCTION

It has been suggested that the decreased urine flow and sodium excretion rate observed in response to low doses of AII can be explained by the concomitant changes in renal haemodynamics (Navar and Langford, 1974). However, Barraclough et al., (1967) and Johnson and Malvin, (1977) reported that the antinatriuretic response to infusion of low doses of AII was independent of changes in GFR and suggested that low doses of the peptide may directly stimulate tubular reabsorption.

The direct effect of AII on proximal tubular reabsorption has been investigated using in vivo micropuncture techniques and isolated perfused tubule preparations. Using in vivo micropuncture techniques in rats, Harris and Young, (1977) and Spinelli and Walther (1979) demonstrated that low doses of AII (10^{-12}M - 10^{-10}M) directly stimulated proximal tubular sodium and fluid reabsorption. In addition, Schuster et al., (1984) found that low doses (10^{-11}M) of the peptide stimulated fluid reabsorption by isolated segments of rabbit proximal convoluted tubule.

Therefore, it is possible that the antidiuretic and antinatriuretic response to low doses of AII may be due to a direct stimulatory effect of the peptide on proximal tubular reabsorption. To evaluate this possibility, experiments were carried out to determine the effects of systemic infusion of low doses of AII on proximal tubular fluid reabsorption and whole kidney function in the anaesthetised rat.

METHODS

ANIMALS

All experiments were performed on male Wistar rats (250-300g) allowed access to tap water ad libitum. The rats were anaesthetised and surgically prepared for micropuncture as described in Section 2.

EXPERIMENTAL PROCEDURE

After placement of the jugular cannulae, a priming injection of 1 ml of 0.9% saline was given to replace fluid losses during surgery, and 0.9% saline was infused via both cannulae (total rate of 75 $\mu l.min^{-1}$). Upon completion of surgery, a priming dose of 1ml of 2% Inutest was administered through one of the jugular cannulae and then a solution of 2% Inutest infused throughout the remainder of the experiment. Saline was infused from the other syringe. A period of 90 minutes was then allowed for the clearance marker to reach a steady state and for stabilisation of renal function.

Three groups of experiments were performed.

Group 1 - Infusion of 0.1 $ng.min^{-1}$ AII (n = 6)

The equilibration period was followed by a 60 minute period during which 0.9% saline and Inutest were infused. AII was then added to the syringe previously used for infusion of saline alone and infused at a rate of 0.1 $ng.min^{-1}$ for one hour. There then followed a 60 minute recovery period during which only saline and Inutest were infused.

Group 2 - Infusion of 1, 5 and 10 $ng.min^{-1}$ AII (n = 7)

The 90 minute equilibration was followed by a protocol consisting of 5 periods each of 60 minutes. During the first period (control) saline and Inutest were both infused, as in group 1. In 3 subsequent

periods, AII was administered from the saline syringe, at doses of 1, 5 and 10 ng.min^{-1}. The final hour was a recovery period during which saline and Inutest only were infused.

Group 3 - Time controls (n = 5)

The protocol used in this group differed slightly from that employed in groups 1 and 2 in that ^{14}C inulin (Amersham) was used instead of Inutest as the clearance marker for GFR. After completion of surgery a priming dose of 1 ml (containing 4.6 KBq - 0.124 µCi) of ^{14}C inulin was administered through one of the jugular cannulae. ^{14}C inulin was then infused (37.5 µl.min^{-1}) continuously throughout the remainder of the experiment at the rate of 344 Bq.min^{-1} (9.3 x 10^{-3} µCi.min^{-1}). As in groups 1 and 2, a 90 minute equilibration period was then allowed for the attainment of steady state conditions. This was followed by five consecutive 60 minute periods during which only saline and ^{14}C inulin were infused (each at 37.5 µl.min^{-1}).

In all groups, the total rate of fluid administration remained constant (75 µl.min^{-1}). At the mid-point of each 60 minute urine collection period, an arterial blood sample (400 µl) was taken and an aliquot used for determination of haematocrit. The cells were separated by centrifugation and the plasma removed. During each 60 minute urine collection period, randomly selected surface proximal tubules were micropunctured. Proximal tubular fluid reabsorption (Jv) was determined using the shrinking droplet technique (Gyory, 1971). At the end of each experiment, the kidney was removed, blotted dry and weighed.

In Fig. 12-20, statistical significance is indicated as:
*, $P<0.05$ when compared to control period; calculated from paired data using two-way analysis of variance and Duncan's multiple range test.

RESULTS

MEAN ARTERIAL BLOOD PRESSURE

The results for MABP are shown in Fig. 12. In all groups, MABP remained above 100 mmHg throughout the course of the experiment. There were no significant changes in MABP during the time control experiments (group 3). Infusion of AII at rates of 0.1,1,5 and 10ng.min^{-1} did not significantly increase MABP. However, time-dependent decreases in arterial blood pressure occurred in both groups 1 and 2. The reasons for these time-related decreases in blood pressure remain unclear.

URINE FLOW

Data obtained for urine flow are shown in Fig. 13. There were no significant differences in the control period values for urine flow rate between the three groups of rats (one-way analysis of variance). In the time control experiments (group 3), urine flow rate was 18.77 ± 2.93 µl.min^{-1} during the second 60 minute infusion period, a value not significantly different from that during the first 60 minute infusion period (16.79 ± 3.29 µl.min^{-1}). However, urine flow increased significantly to 21.15 ± 3.73, ($P<0.05$), 22.12 ± 4.19 ($P<0.05$) and 20.72 ± 4.01 µl.min^{-1} ($P<0.05$) during the last three 60 minute infusion periods respectively.

In group 1 (low dose AII) urine flow did not change significantly during infusion of 0.1 ng.min^{-1} AII. The pattern of urine flow during the 0.1 ng.min^{-1}AII infusion (group 1) experiments was not significantly different from that during the first three 60 minute infusion periods in the time control (group 3) experiments. However, the pattern of urine flow during the control, 1,5 and 10 ng.min^{-1} AII infusion periods in group 2 was significantly different ($P<0.05$) from that during the first four 60 minute infusion periods in the time control experiments (group 3). Indeed, the increase in urine flow observed in the time control

FIG 12 THE EFFECT OF ANGIOTENSIN II ON MEAN ARTERIAL BLOOD PRESSURE

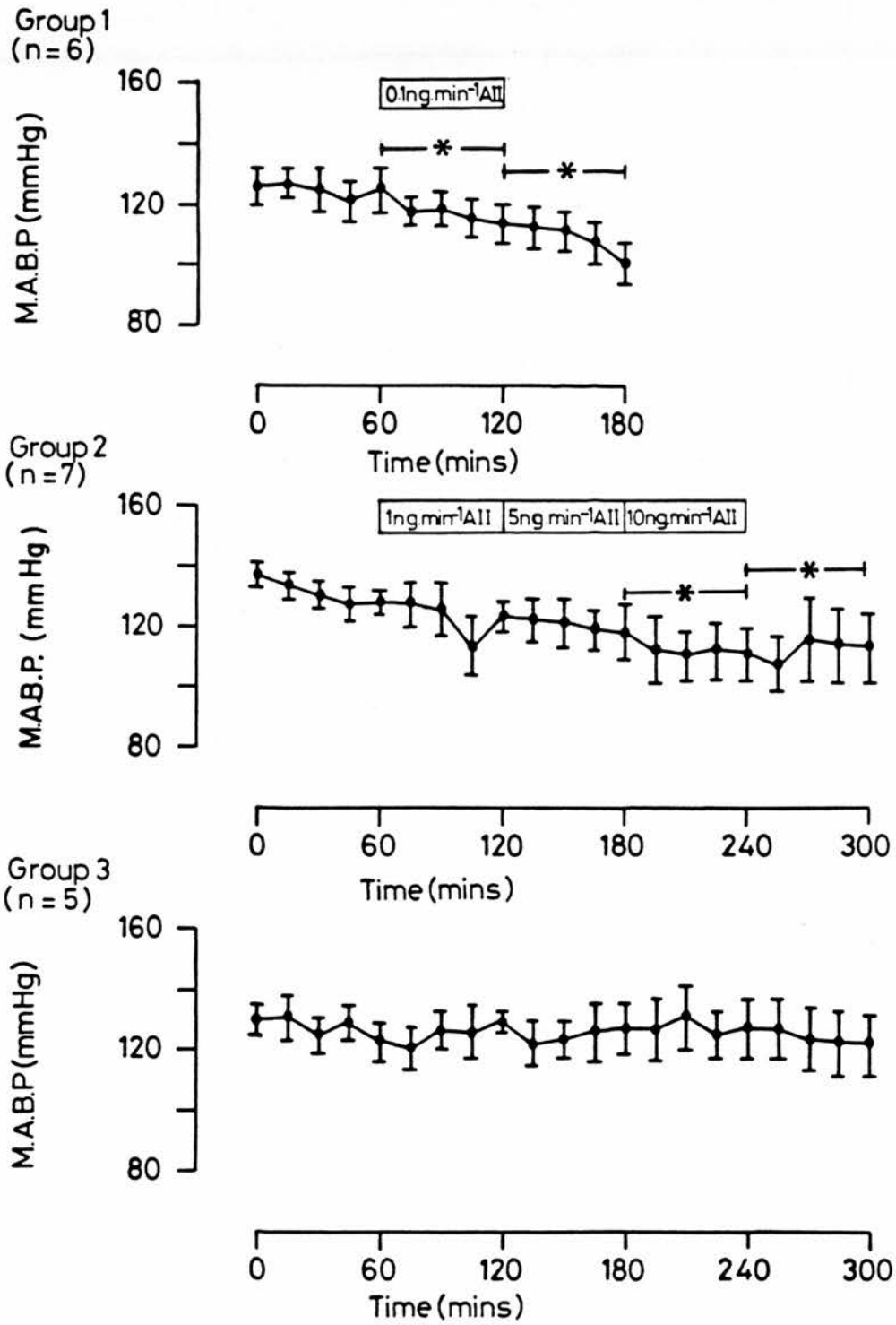
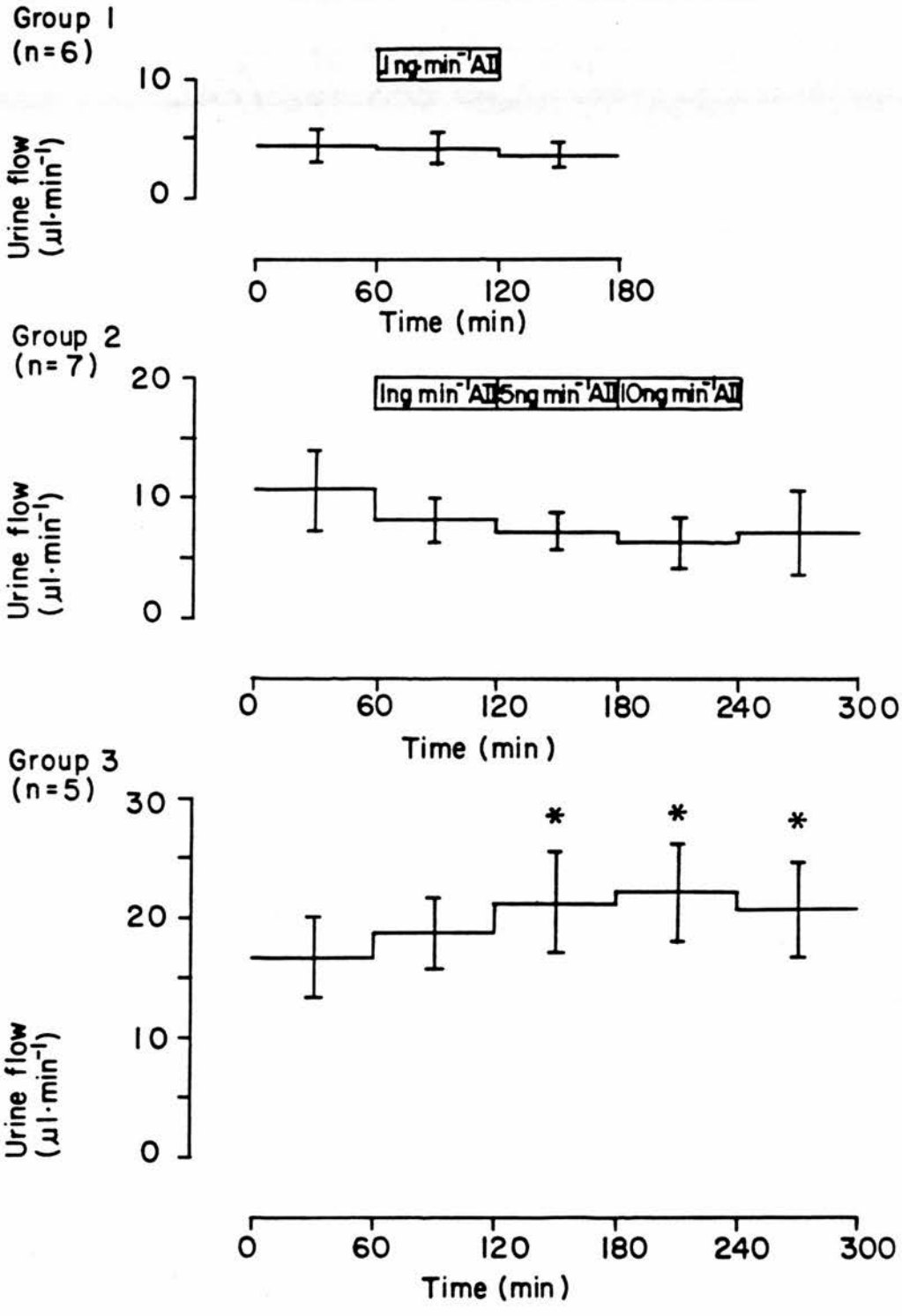


FIG 13 THE EFFECT OF ANGIOTENSIN II ON MEAN URINE FLOW RATE



experiments (group 3) did not occur in group 2 (high doses AII). Thus, infusion of AII at rates of 5 and 10 ng.min^{-1} resulted in an antidiuresis when the effects of the peptide were compared to the effects of infusion of saline alone.

GLOMERULAR FILTRATION RATE (GFR)

Due to technical difficulties, accurate measurements of GFR were not obtained for the group 1 (low dose AII) experiments. Glomerular filtration rate did not change significantly during the time control (group 3) experiments. In group 2 (high doses AII) glomerular filtration rate was not significantly altered during infusion of 1 and 5 ng.min^{-1} AII, but decreased significantly to 0.58 ± 0.07 ml.min^{-1}.g^{-1} ($P<0.05$) during infusion of 10 ng.min^{-1} AII. During the recovery period, glomerular filtration rate increased to 0.94 ± 0.2 ml.min^{-1}g^{-1}, a value not significantly different from control. These results are shown in Fig. 14.

SODIUM EXCRETION RATE (Fig. 15)

There were no significant differences in the control period values for sodium excretion between the three groups of rats (one-way analysis of variance). In the time control experiments (group 3), the mean sodium excretion rate during the second 60 minute infusion period was 2.14 ± 0.39 μmol. min^{-1}, a value not significantly different from that during the first 60 minute infusion period (1.73 ± 0.33 μmol.min^{-1}). However, sodium excretion increased to 2.79 ± 0.56 ($P<0.05$), 3.45 ± 0.81 ($P<0.05$) and 3.38 ± 0.81 μmol.min^{-1} ($P<0.05$) during the last three 60 minute infusion periods respectively.

In group 1 (low dose AII) sodium excretion rate did not change significantly during infusion of 0.1 ng. min^{-1} AII. The pattern of sodium excretion during the 0.1 ng.min^{-1} AII infusion (group 1) experiments was not significantly different from that during the first three

FIG 14 THE EFFECT OF ANGIOTENSIN II ON MEAN GLOMERULAR FILTRATION RATE

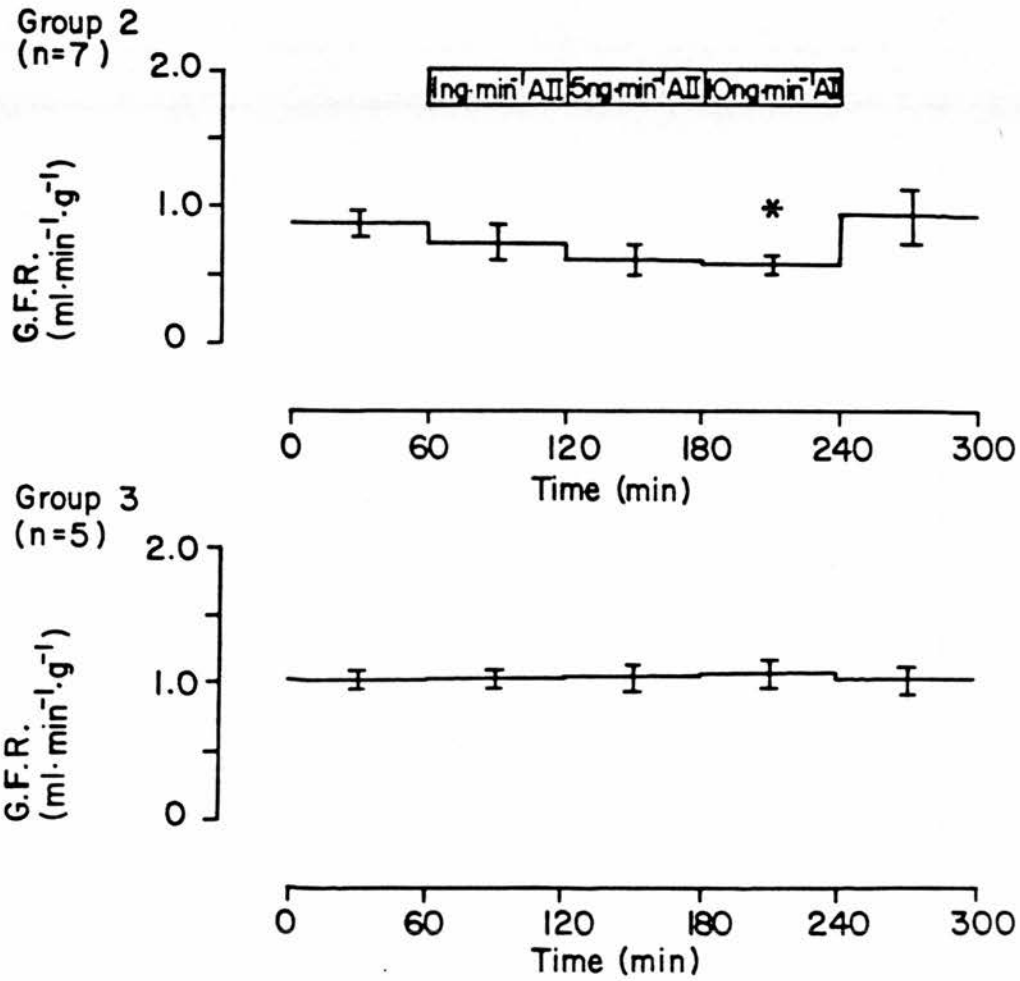
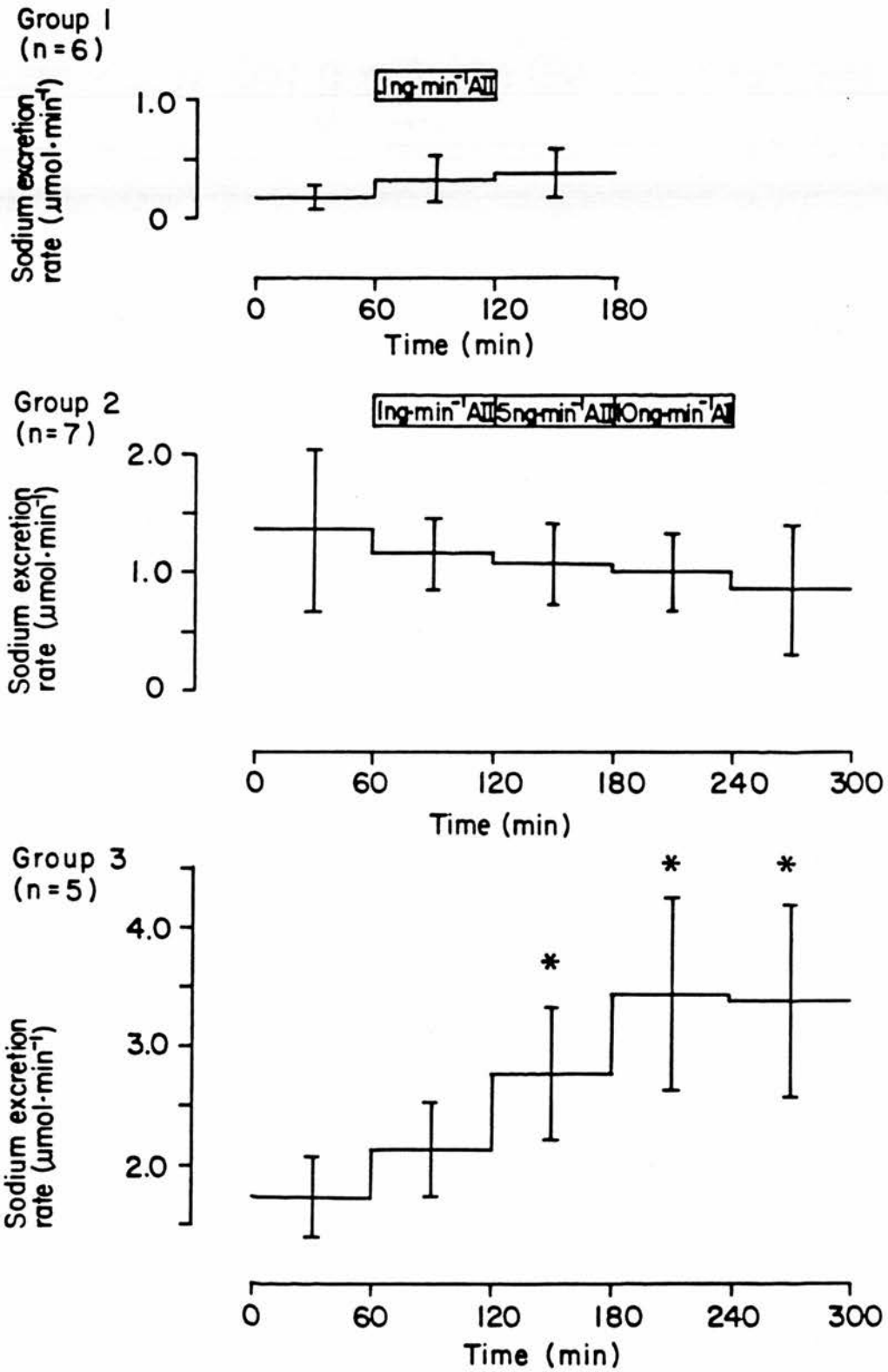


FIG 15 THE EFFECT OF ANGIOTENSIN II ON MEAN SODIUM EXCRETION RATE



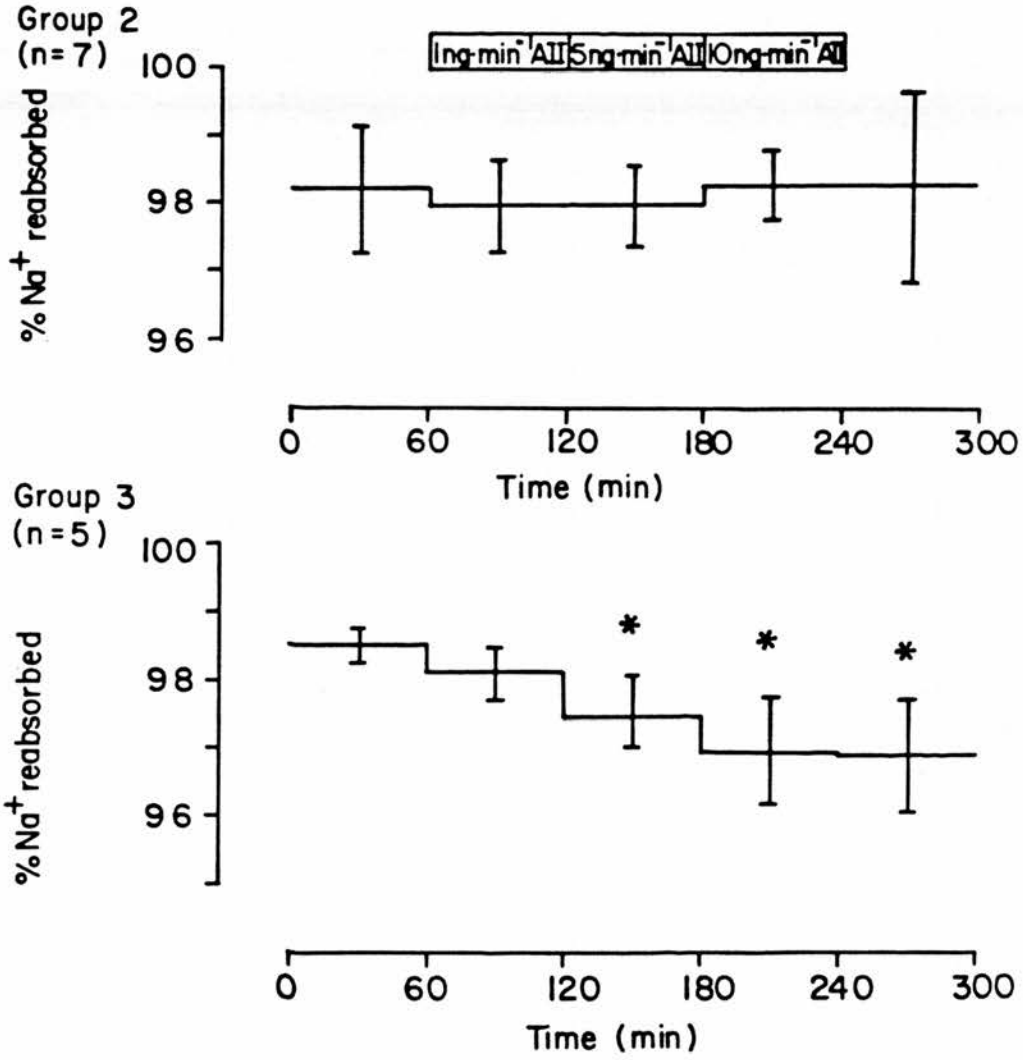
60 minute infusion periods in the time control (group 3) experiments. However, the pattern of sodium excretion during the control 1,5 and 10 $ng.min^{-1}$ AII infusion periods in group 2 was significantly different ($P<0.05$) from that during the first four 60 minute infusion periods in time control (group 3) experiments. Indeed, the increase in sodium excretion observed in the time control experiments (group 3) did not occur in group 2. Thus, infusion of AII at rate of 5 and 10 ng. min^{-1} resulted in an antinatriuresis when the effects of the peptide were compared to the effects of infusion of saline alone. However, at no time in group 2 did infusion of AII elicit a significant reduction in sodium excretion compared with the initial control value.

PERCENTAGE OF FILTERED SODIUM WHICH WAS REABSORBED

Mean values for this parameter are shown in Fig. 16. In the time control experiments (group 3), the percentage of filtered sodium which was reabsorbed did not vary significantly during the first two 60 minute periods. However, a significant reduction from 98.51 ± 0.25% during the first 60 minute period to 97.49 ± 0.56, 96.97 ± 0.78 and 96.90 ± 0.83% during the last three 60 minute periods respectively was observed ($P<0.05$ in all cases).

As accurate measurements of GFR were not obtained for the group 1 (low dose AII) experiments, it was not possible to calculate fractional sodium reabsorption values for this group. In group 2 (high doses AII), the percentage of filtered sodium which was reabsorbed was unaffected by the AII infusion rates employed. Thus, the progressive decrease in fractional sodium reabsorption observed in the time control experiments (group 3) did not occur in group 2 (high doses AII).

FIG 16 THE EFFECT OF ANGIOTENSIN II ON THE MEAN PERCENTAGE OF FILTERED SODIUM WHICH WAS REABSORBED



POTASSIUM EXCRETION RATE

The mean values for this parameter are shown in Fig. 17. No significant changes in the absolute excretion of potassium were observed in any of the three groups.

PERCENTAGE OF FILTERED POTASSIUM WHICH WAS EXCRETED

As accurate measurements of GFR were not obtained for the group 1 (low dose AII) experiments, it was not possible to calculate fractional potassium excretion values for this group of experiments. No significant changes in fractional potassium excretion occurred in either group 2 or 3 (Fig. 18).

HAEMATOCRIT

These results are shown graphically in Fig. 19. Haematocrit did not vary significantly in the 0.1 $ng.min^{-1}$ AII experiments (group 1). In group 2, arterial haematocrit determined during the 1 $ng.min^{-1}$ AII infusion period (47.8 ± 0.7%) did not differ significantly from the control value (48.3 ± 0.8%). However, haematocrit decreased significantly to 45.4 ± 0.6% ($P<0.05$) during the 5 $ng.min^{-1}$ AII period, to 43.1 ± 0.7% ($P<0.05$) during the 10 $ng.min^{-1}$ AII period and to 41.6 ± 0.9% ($P<0.05$) during the recovery period.

In the time control experiments (group 3), arterial haematocrit did not vary significantly during the first two 60 minute periods. A significant reduction from 46.3 ± 1.4% during the first period to 43.9 ± 1.0 ($P<0.05$), 42.4 ± 1.2 ($P<0.05$) and 41.3 ± 0.4% ($P<0.05$) during the last three hours of infusion was observed. The overall patterns of arterial haematocrit, at any given time, were not significantly different for the three groups of rats.

FIG 17 THE EFFECT OF ANGIOTENSIN II ON MEAN POTASSIUM EXCRETION RATE

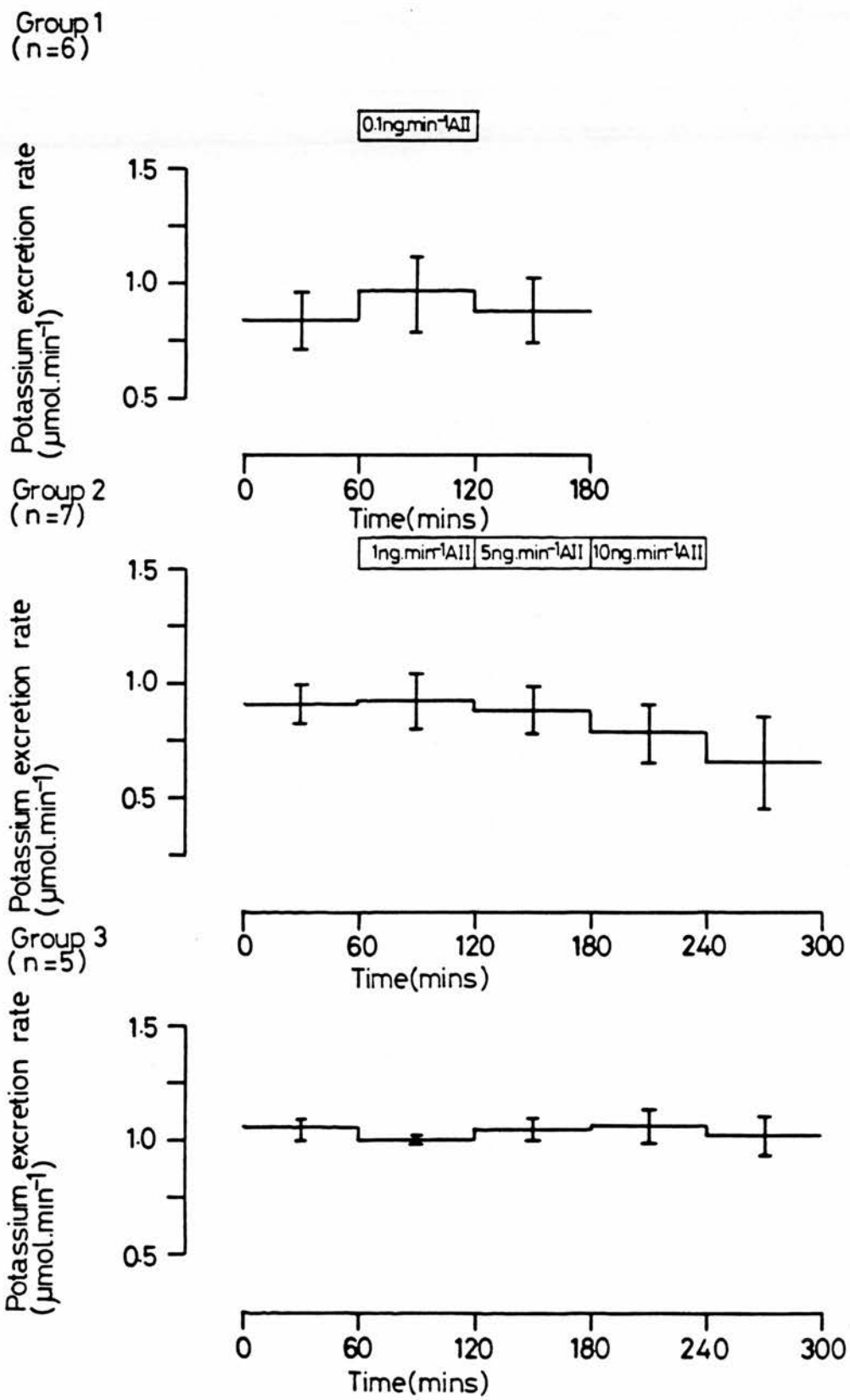


FIG 18 THE EFFECT OF ANGIOTENSIN II ON THE MEAN PERCENTAGE OF FILTERED POTASSIUM WHICH WAS EXCRETED

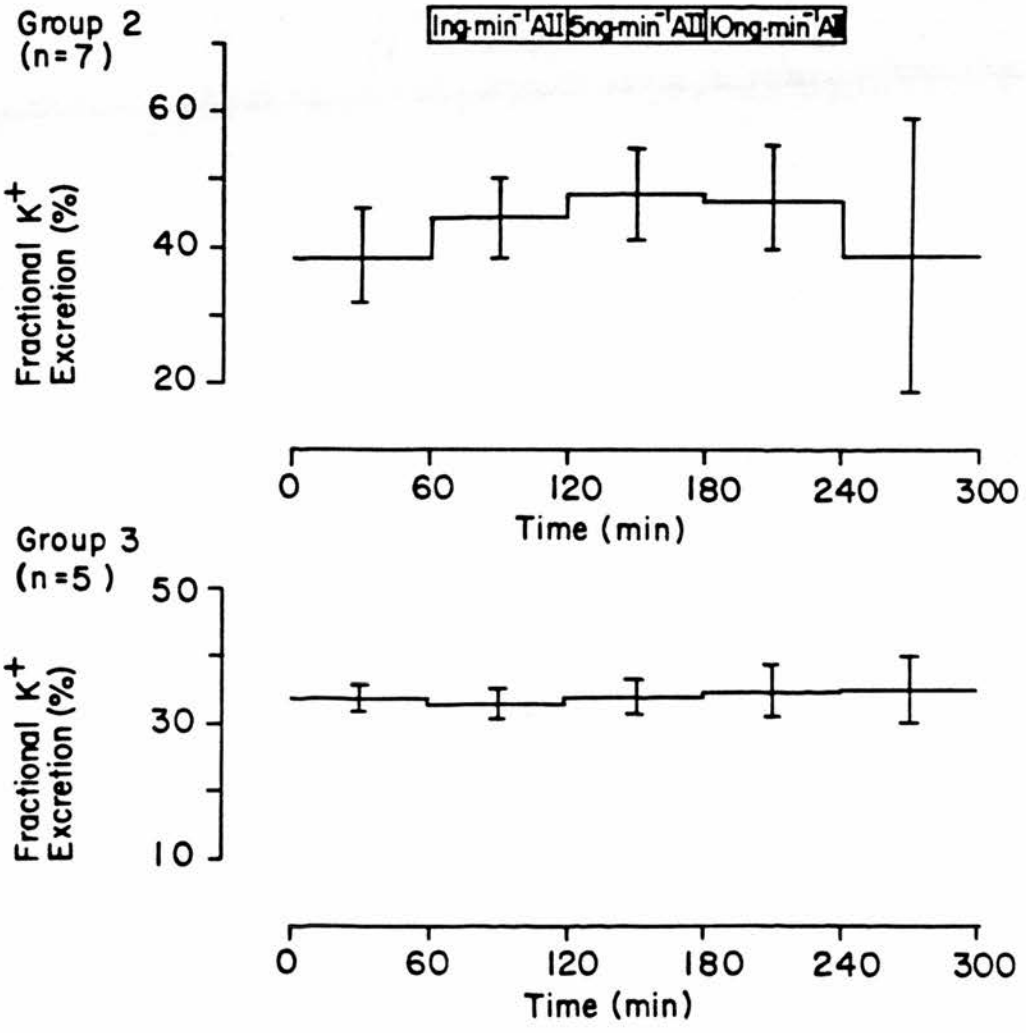
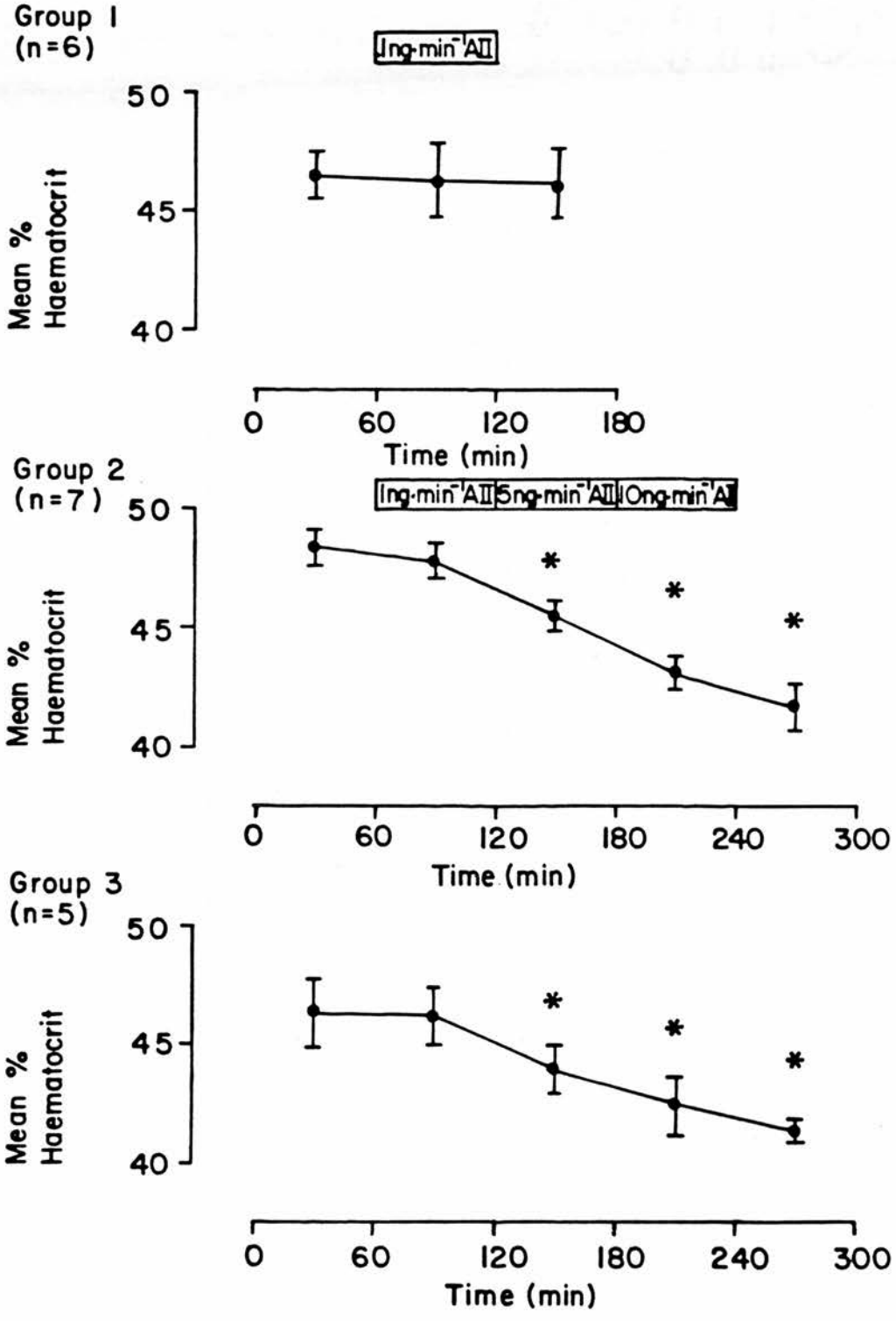


FIG 19 THE EFFECT OF ANGIOTENSIN II ON MEAN ARTERIAL HAEMATOCRIT

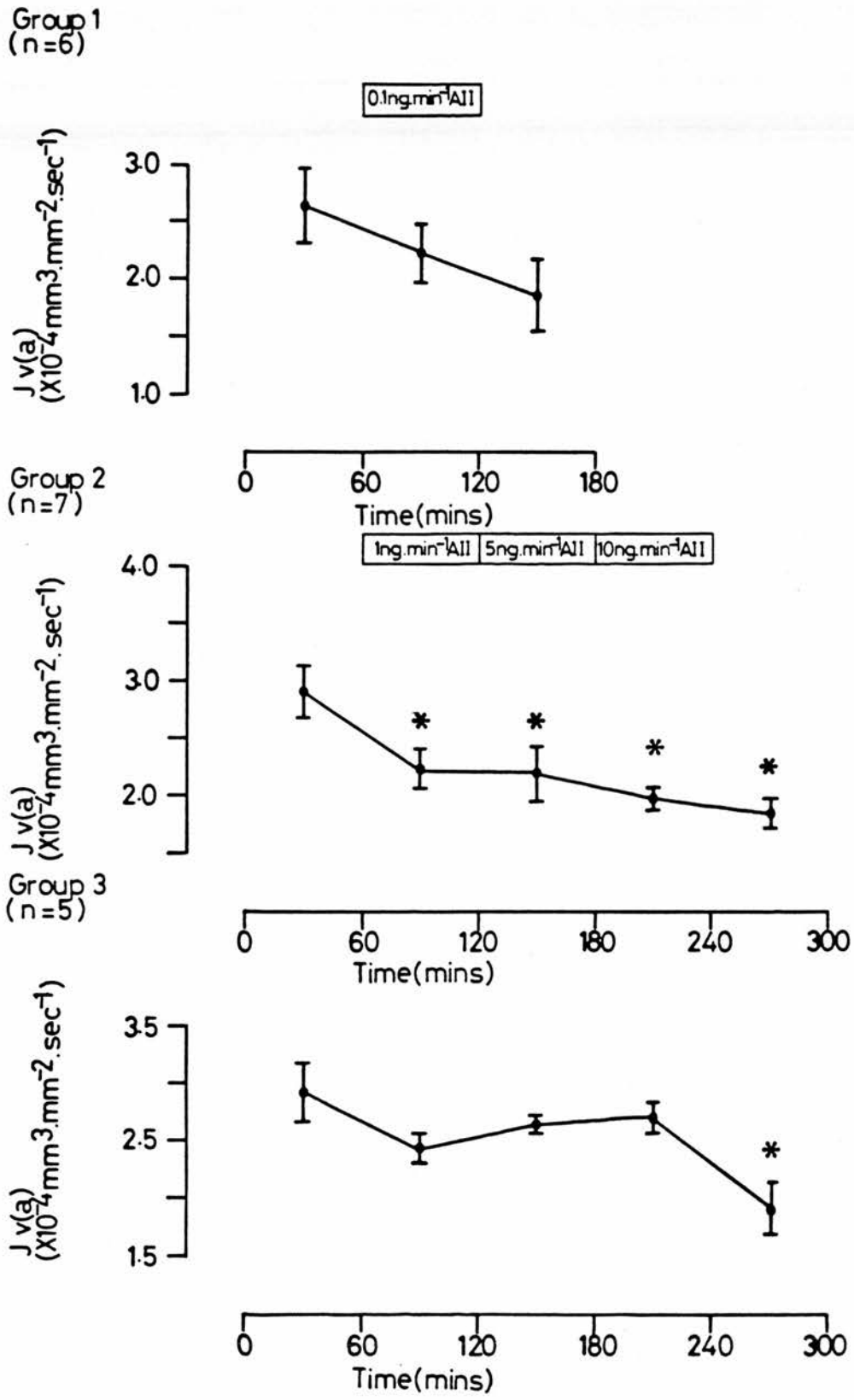


PROXIMAL TUBULAR FLUID REABSORPTION (Jv)

Data obtained for mean Jv are presented in Fig. 20. In the time control experiments (group 3), mean Jv during the first 60 minute infusion period was 2.93 ± 0.26 x 10^{-4} $mm^{-3}.mm^{-2}.sec^{-1}$. Mean Jv did not vary significantly during the first four 60 minute infusion periods, but decreased significantly to 1.92 ± 0.22 x 10^{-4} $mm^{3}.mm^{-2}.sec^{-1}$ ($P<0.05$) during the fifth 60 minute infusion period. Infusion of AII at rates of 0.1,1,5 and 10 $ng.min^{-1}$ did not increase proximal tubular fluid reabsorption. In group 1, (low dose AII), mean Jv did not vary significantly during the experiment. The pattern of proximal Jv during the 0.1 $ng.min^{-1}$ AII infusion (group 1) experiments was not significantly different from that during the first three 60 minute infusion periods in the time control (group 3) experiments.

In group 2 (high doses AII), the values obtained for mean Jv during the 1,5 and 10 $ng.min^{-1}$ AII infusion periods were 2.24 ± 0.18, 2.20 ± 0.24 and 1.99 ± 0.10 x 10^{-4} $mm^{3}.mm^{-2}.sec^{-1}$ respectively. Although these values were significantly lower than the initial control value (2.91 ± 0.23 x 10^{-4} $mm^{3}.mm^{-2}.sec^{-1}$, $P<0.05$ in all cases) they were not significantly different from the recovery period value (1.85 ± 0.13 x 10^{-4} $mm^{3}.mm^{-2}.sec^{-1}$). Indeed, mean Jv during the recovery period was significantly lower than during the initial control period ($P<0.05$) Furthermore, the pattern of proximal Jv during the control, 1,5 and 10 $ng.min^{-1}$ AII infusion periods in group 2 was not significantly different from that during the first four 60 minute infusion periods in the time control (group 3) experiments.

FIG 20 THE EFFECT OF ANGIOTENSIN II ON MEAN PROXIMAL TUBULAR FLUID REABSORPTION (J v(a))



DISCUSSION

The present study was designed to determine the effects of systemic infusion of small (sub-pressor) doses of AII on renal function, particularly proximal tubular fluid reabsorption, in the rat.

In the time control experiments (group 3) arterial blood pressure remained stable for the duration of the five hour experiment. However, in groups 1 and 2, arterial blood pressure was found to decrease progressively with time. The reasons for these observed reductions in arterial blood pressure remain unclear. In the time control experiments (group 3) both urine flow and sodium excretion increased during the course of the experiment. These changes occurred in the absence of significant alterations in arterial blood pressure or glomerular filtration rate. The increase in urinary sodium excretion was associated with a progressive decrease in the percentage of filtered sodium which was reabsorbed. Although both urine flow and sodium excretion increased during the time control experiments both absolute and fractional potassium excretion rates did not vary during the course of the experiment. Proximal tubular fluid reabsorption remained stable for the first four hours of infusion but decreased significantly during the fifth 60 minute infusion period (group 3, Fig. 20). Arterial haematocrit was significantly reduced during the infusion of 5 and 10 $ng.min^{-1}$ AII. However, the finding that arterial haematocrit remained significantly decreased during the final recovery period in group 2 and was significantly reduced during the last three hours of infusion in the time control experiments (group 3), suggests that the reductions observed in this parameter were due to a time-dependent effect of systemic infusion of isotonic saline.

Infusion of AII at a rate of $0.1ng.min^{-1}$ (group 1) did not significantly alter any of the parameters of renal function measured. Indeed,

the patterns of urine flow, urinary sodium and potassium excretion and proximal Jv during the 0.1 ng.min^{-1} AII infusion experiments (group 1) were not significantly different from those during the first three 60 minute infusion periods in the time control experiments (group 3). In group 2, the effects of infusion of AII on urine flow and urinary sodium excretion were signifciantly different compared to the effects of infusion of saline alone (group 3). Indeed the patterns of urine flow and urinary sodium excretion during the control, 1, 5 and 10 ng.min^{-1} AII infusion periods (group 2) were significantly different from those during the first four 60 minute infusion periods in the time control experiments (group 3). The significant increase in urine flow and sodium excretion that occurred during the last three 60 minute infusion periods in group 3 did not occur in group 2. Thus, infusion of AII at rates of 5 and 10 ng. min^{-1} resulted in an antidiuresis and antinatriuresis when the effects of the peptide were compared to the effects of infusion of saline alone. However, these effects of AII were subtle and at no time in group 2 did AII reduce urine flow or urinary sodium excretion to levels significantly lower than the initial (ie, pre-AII) control values.

In addition to this subtle antidiuretic and antinatriuretic effect of AII, infusion of the peptide at a rate of 10 ng.min^{-1} significantly reduced GFR (Fig. 14). Proximal tubular fluid reabsorption during the 1, 5 and 10 ng.min^{-1} AII infusion periods (group 2) was significantly lower than the initial control value. However, proximal tubular fluid reabsorption did not return to control levels following infusion of these three sub-pressor doses of AII, but remained significantly reduced, suggesting that the observed reductions in proximal Jv were not due to an inhibitory effect of AII, but represented time-dependent decreases in this parameter. Indeed, the effect of these three subpressor doses of

the peptide on proximal reabsorption was not significantly different to the effect of infusion of saline alone (group 3).

Thus, the results from the present study are consistent with the possibility that the antidiuretic and antinatriuretic effects of systemic infusion of 10 ng.min^{-1} AII are due, at least in part, to altered renal haemodynamics (ie, a reduction in GFR). In addition, the present findings do not support the concept that the antinatriuretic effect of systemic AII is due to facilitation, by the peptide, of proximal tubular fluid reabsorption. Indeed, in the present study, infusion of AII at rates of 0.1,1,5 and 10 ng.min^{-1} did not increase proximal tubular fluid reabsorption. However, the results from the present experiments do not rule out the possibility that systemic AII may stimulate tubular reabsorption by more distal segments of the nephron.

It is possible that the decrease in GFR observed during infusion of 10 ng.min^{-1} AII (Fig. 14) was due to the associated reduction in MABP and not to a direct action of the peptide. However, during the recovery period, GFR returned to control levels despite the fact that MABP remained significantly reduced, suggesting that the observed reduction in GFR was due to the effect of AII and not the result of a reduction in MABP.

Barraclough et al (1967) suggested that in its normal state, the rat produces almost optimal antinatriuretic amounts of AII and that elevation of the plasma AII concentration will not lead to a reduction in sodium excretion. Also, they suggested that it may be necessary to sodium load the animal to suppress endogenous AII production in order to demonstrate an antinatriuretic effect of infusion of low doses of the peptide. This may explain why only very subtle antidiuretic and antinatriuretic effects of AII were observed in the present study.

It has been demonstrated that AII exerts a direct stimulatory effect on proximal tubular sodium and fluid reabsorption, with a maximum

effect at a peritubular concentration of approximately 10^{-11}M (Harris and Young, 1977; Spinelli and Walther, 1979; Schuster et al., 1984). This concentration is within the range found by radioimmunoassay in normal rat plasma (Oster et al., 1973; Semple and Morton, 1976; Brown et al., 1981). These results together with the finding by Harris et al., (1984) that infusion of captopril in the anaethetised rat prepared for micropuncture results in a 30% reduction in proximal tubular fluid reabsorption suggest that the normal rat (ie, not sodium loaded or depleted) produces amounts of AII that are almost optimal for maximal stimulation of proximal tubular fluid reabsorption and sodium retention. It may therefore be necessary to suppress endogenous angiotensin production in order to demonstrate a stimulatory effect on proximal tubular fluid reabsorption and a pronounced antinatriuretic effect of infusion of exogenous hormone.

SECTION 5

THE EFFECT OF INFUSION OF SUB-PRESSOR DOSES OF ANGIOTENSIN II ON RENAL FUNCTION IN SODIUM LOADED RATS

INTRODUCTION

Barraclough et al., (1967) suggested that the rat normally produces amounts of angiotensin that are optimal for sodium retention and that it is probably necessary to suppress endogenous angiotensin production in order to demonstrate an antinatriuretic effect of the infused hormone. As discussed previously (Section 4), it is possible that this may explain the failure to observe a stimulation of proximal reabsorption and a pronounced antinatriuresis in response to infusion of low doses of AII in the experiments described in the previous section.

Plasma renin activity has been shown to vary inversely with dietary sodium intake in all species examined (Brown et al., 1963;1964b; Davis and Freeman, 1976; Sancho et al., 1976: Wong and Zimmerman, 1982). Alterations in dietary sodium intake have been used to alter the activity of the renin-angiotensin system. Sodium deprivation markedly increases plasma renin activity, kidney renin concentration and plasma AII levels while increased salt intake (sodium loading) lowers kidney renin concentration and plasma renin activity, ie, supresses the renin-angiotensin system (Gavras et al., 1973; Davis and Freeman, 1976; Mendelsohn, 1976; 1979; Fray et al., 1977; Semple, 1981). However, intrarenal AII concentrations have been found not to be suppressed to the same extent as renal tissue renin concentrations during salt loading (Mendelsohn, 1979; 1982). Nevertheless, the basic hypothesis is that the activity of the renin-angiotensin system is augmented by sodium deprivation and suppressed by sodium loading.

The combination of a high salt diet with the administration of a mineralocorticoid such as desoxycorticosterone acetate (DOCA) has been used to deplete kidneys of renin and suppress circulating AII levels (Potkay and Gilmore, 1973; Kaloyanides et al., 1974; Hall et al., 1977a).

Alternative methods of suppressing the activity of the renin-angiotensin system have involved the use of pharmacological inhibitors or antagonists that either block the conversion of AI to AII or directly interfere with the action of AII (Hall et al., 1977b; 1979 b; Fagard et al., 1978; Thurston and Swales, 1978; Hollenberg, 1979; Ploth et al., 1979; McCaa, 1982; Navar et al., 1982; Ploth and Roy, 1982 a; Wong and Zimmerman, 1982).

The following experiments were designed to investigate the effects of systemic infusion of low (sub-pressor) doses of AII on proximal tubular fluid reabsorption and whole kidney function in anaesthetised sodium loaded rats. Rats were sodium loaded in an attempt to suppress endogenous angiotensin production and thus facilitate the demonstration of a stimulation of proximal tubular fluid reabsorption and a pronounced antinatriuresis in response to infusion of AII.

METHODS

ANIMALS

Studies were performed on sodium loaded male Wistar rats (250 -325g) maintained on a standard rat diet. Two groups of animals were examined using the protocols described below.

Group 1 (n = 5)

Experiments were carried out on rats loaded with sodium by replacing their drinking water with 0.9% saline for 8 - 10 days prior to the experiment. In this group of animals, the effect on renal function of infusion of AII, at a rate of 0.1 $ng.min^{-1}$, was determined. However, as is shown in the results section, this dose of AII failed to alter any parameter of renal function measured. This may have been due to the fact that the dose of AII used was too low to elicit an antinatriuresis and/or that the animals were insufficiently sodium loaded to suppress endogenous angiotensin production and thereby allow the exogenous hormone to elicit an antinatriuresis.

Thus, a second group of experiments (group 2) were performed to evaluate the renal effects of infusion of AII, at the rates of 1 and 5 $ng.min^{-1}$, in rats subjected to a more severe form of sodium loading.

Group 2 (n = 5)

In this group, rats were sodium loaded by replacing their drinking water with 2% saline for at least 5 - 6 weeks prior to the experiment.

SURGICAL PROCEDURE

The surgical procedure employed was the same for both group of rats and is described in Section 2.

EXPERIMENTAL PROCEDURE

After placement of the jugular cannulae, a priming injection of 1 ml of 0.9% saline was administered to replace fluid losses during surgery, and 0.9% saline was infused from both syringes (total rate of 75 $\mu l.min^{-1}$). After completion of surgery, a priming dose (1 ml of 2% solution) of Inutest was administered through one of the jugular cannulae and followed by an infusion at 37.5 $\mu l.min^{-1}$ throughout the remainder of the experiment.

A period of at least 90 minutes was allowed for attainment of steady state conditions, and was followed by a 60 minute control period, during which the animals received only saline and Inutest. After the control period, the protocol differed for the two groups of animals.

Group 1

AII was added to the saline syringe and infused for 60 minutes at the rate of 0.1 $ng.min^{-1}$. There then followed a recovery period during which only saline and Inutest were infused.

Group 2

AII was added to the saline syringe and administered at the rates of 1 and 5 $ng.min^{-1}$ for each of the two subsequent 60 minute periods. Again there was a final 60 minute recovery period during which only saline and Inutest were infused.

As the experimental procedure for both groups included a recovery period, it was not considered necessary to perform separate time control experiments.

RESULTS

MEAN ARTERIAL BLOOD PRESSURE (Fig. 21)

There was no significant difference in the initial arterial blood pressure between the two groups of rats. Infusion of AII at rates of 0.1,1 and 5 $ng.min^{-1}$ did not significantly increase arterial blood pressure. However, time-dependent decreases in this parameter occurred in both groups. The reasons for these time-related decreases in blood pressure remain unclear.

URINE FLOW

The results presented in Fig. 22 show that there were no significant alterations in urine flow in either of the two groups of animals.

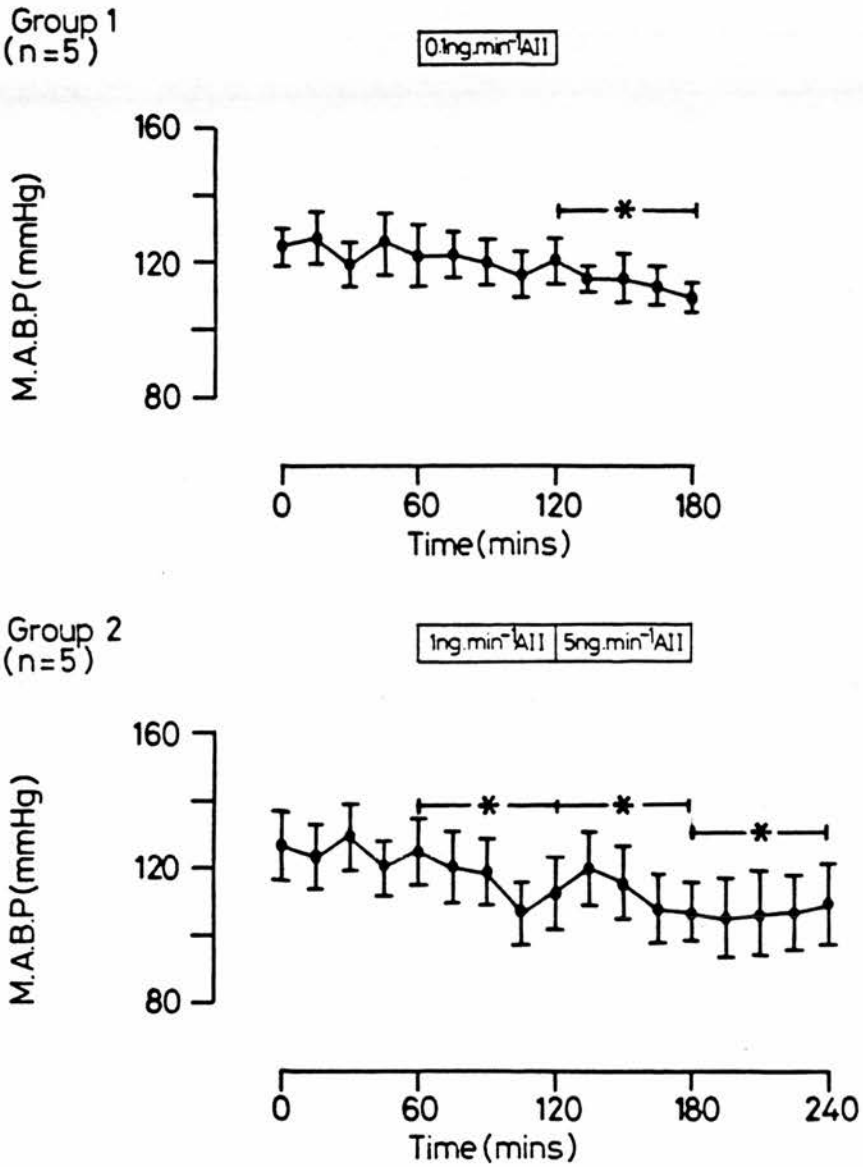
GLOMERULAR FILTRATION RATE (GFR)

Due to technical difficulties, accurate measurements of GFR were not obtained for the group 1 experiments. In group 2, GFR during the 1 $ng.min^{-1}$ AII infusion period was 0.85 ± 0.11 $ml.min^{-1}.g^{-1}$, a value not significantly different from the control value (0.81 ± 0.12 $ml.min^{-1}.g^{-1}$). However, GFR decreased to 0.54 ± 0.08 ($P<0.05$) during infusion of 5 $ng.min^{-1}$ AII and to 0.42 ± 0.07 $ml.min^{-1}.g^{-1}$ ($P<0.05$) during the recovery period. These results are shown in Fig. 23.

SODIUM EXCRETION RATE

As summarised in Fig. 24 no significant change in absolute sodium excretion occurred in either group.

FIG 21 THE EFFECT OF ANGIOTENSIN II ON MEAN ARTERIAL BLOOD PRESSURE



* ,P <0.05 relative to control

FIG 22 THE EFFECT OF ANGIOTENSIN II ON MEAN URINE FLOW RATE

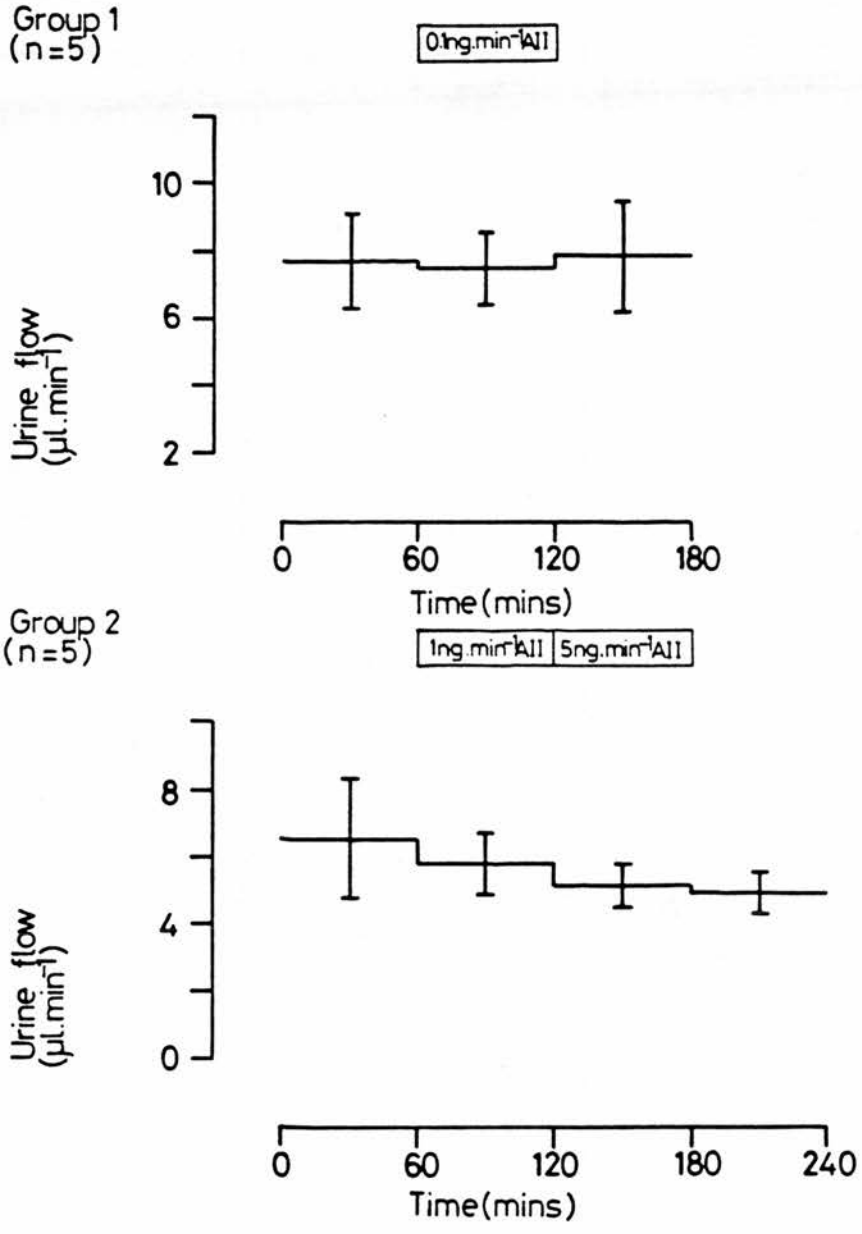
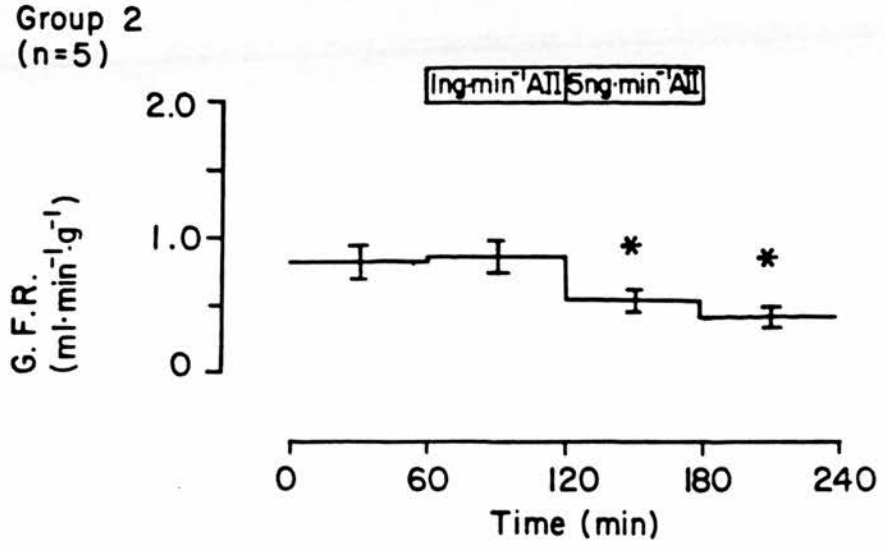


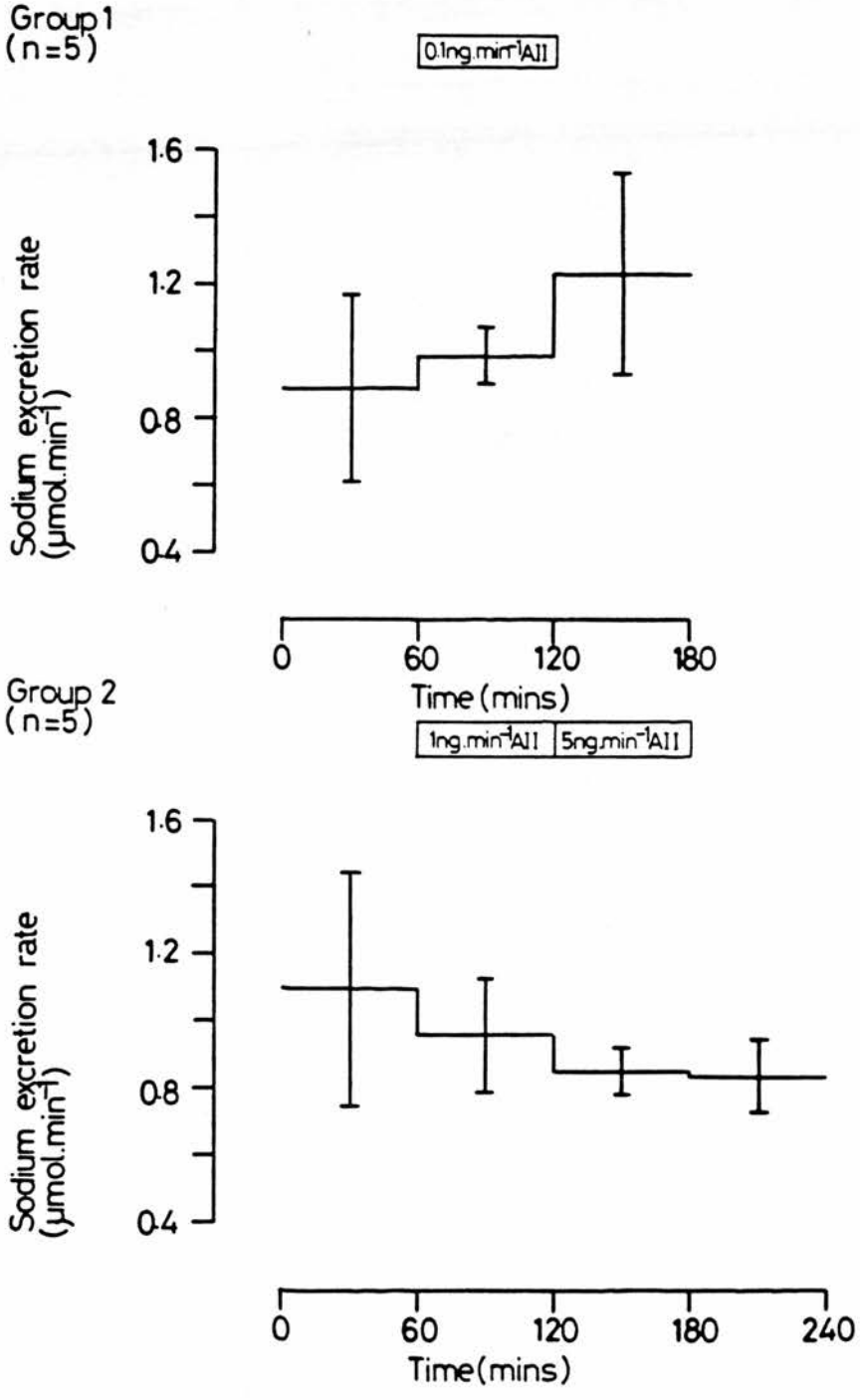
FIG 23 THE EFFECT OF ANGIOTENSIN II ON MEAN GLOMERULAR FILTRATION RATE



* , P<0.05 relative to control

+ , P<0.05 relative to Group I

FIG 24 THE EFFECT OF ANGIOTENSIN II ON MEAN SODIUM EXCRETION RATE



PERCENTAGE OF FILTERED SODIUM WHICH WAS REABSORBED

As accurate measurements of GFR were not obtained for the group 1 experiments, it was not possible to calculate values for fractional sodium reabsorption for this group. As shown in Fig. 25 no significant variation in this parameter occured in group 2.

POTASSIUM EXCRETION RATE (Fig. 26)

The mean potassium excretion rate during the control period in group 1 was significantly higher than the corresponding value in group 2 (0.96 ± 0.18 vs 0.23 ± 0.10 $\mu mol.min^{-1}$ respectively, $P<0.05$). The reason for his difference is not clear. The mean rate at which potassium was excreted was unaffected by any of the doses of AII infused. However, in group 2, mean potassium excretion during the recovery period was 0.32 ± 0.11 $\mu mol.min^{-1}$, a value significantly higher than the control value (0.23 ± 0.10 $\mu mol.min^{-1}$, $P<0.05$).

PERCENTAGE OF FILTERED POTASSIUM WHICH WAS EXCRETED (Fig. 27)

As accurate measurements of GFR were not obtained for the group 1 experiments, it was not possible to calculate values for fractional potassium excretion for this group. In group 2, mean fractional potassium excretion was not significantly altered during infusion of 1 or 5 $ng.min^{-1}$ AII. However, during the recovery period, mean fractional potassium excretion was $15.68 \pm 3.14\%$, a value significantly higher than the control value ($7.02 \pm 1.91\%$, $P<0.05$).

HAEMATOCRIT

The results presented in Fig. 28 show that there was no significant variation in this parameter in either series of experiments.

FIG 25 THE EFFECT OF ANGIOTENSIN II ON THE MEAN PERCENTAGE OF FILTERED SODIUM WHICH WAS REABSORBED

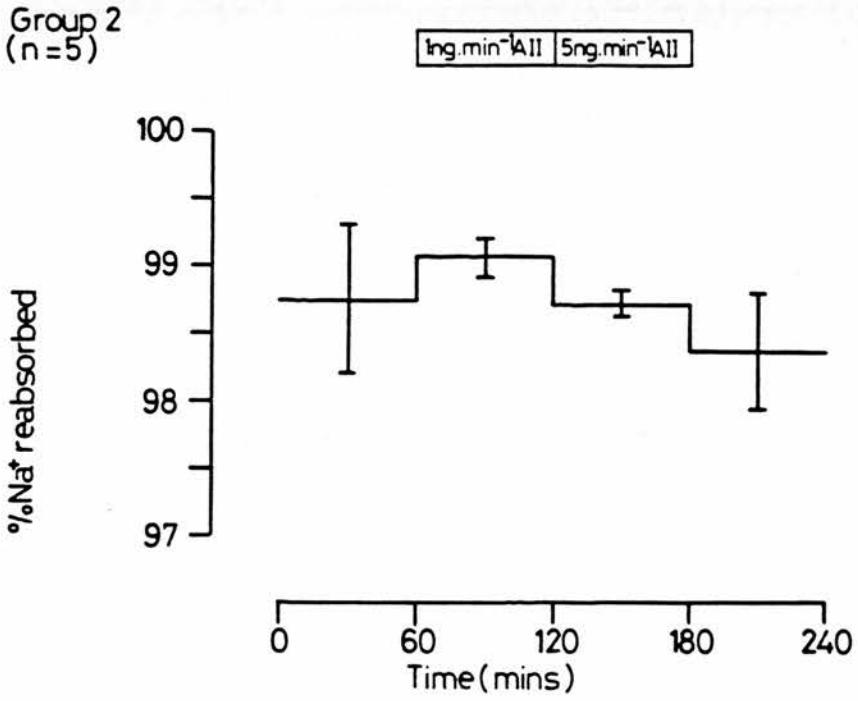
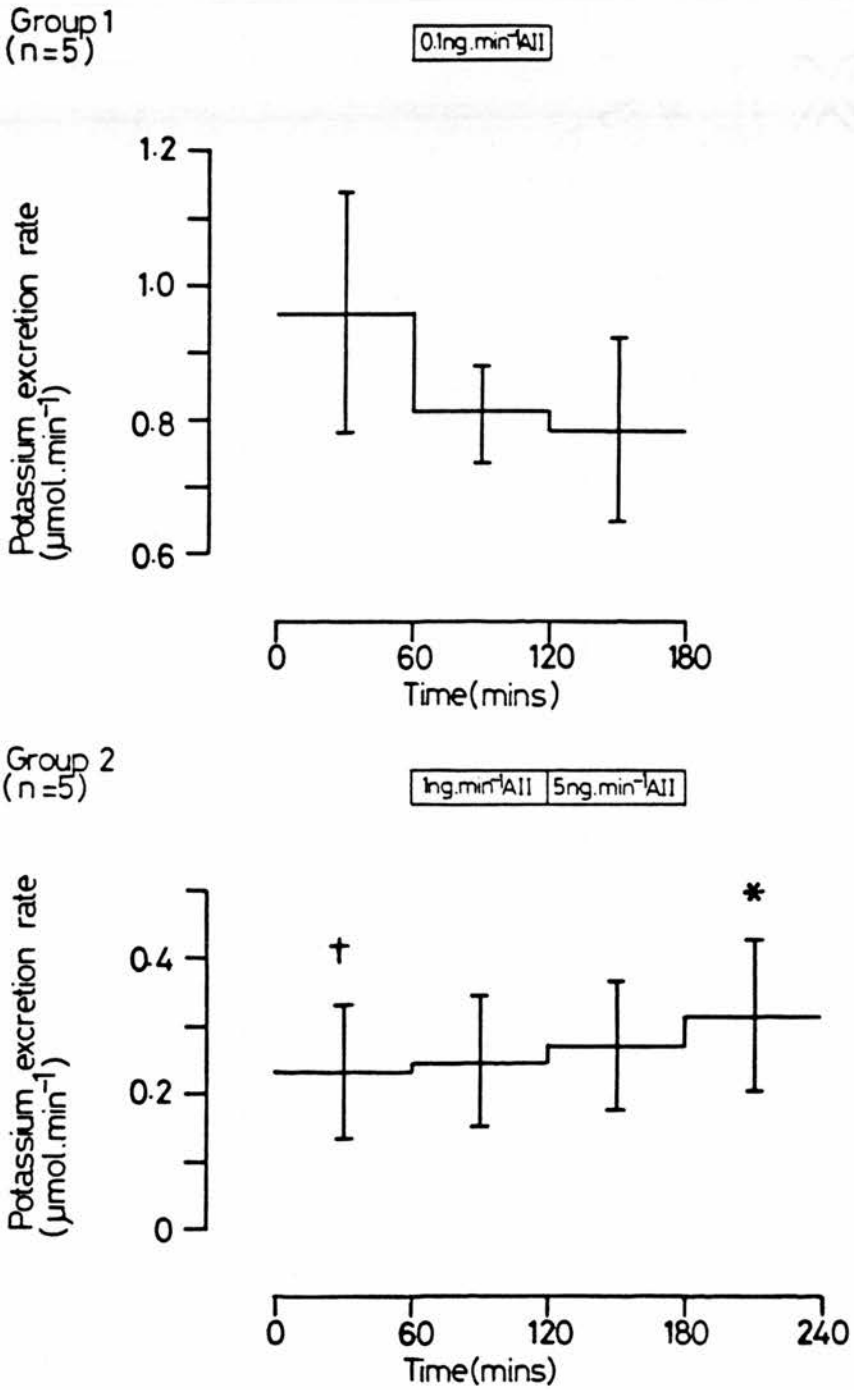
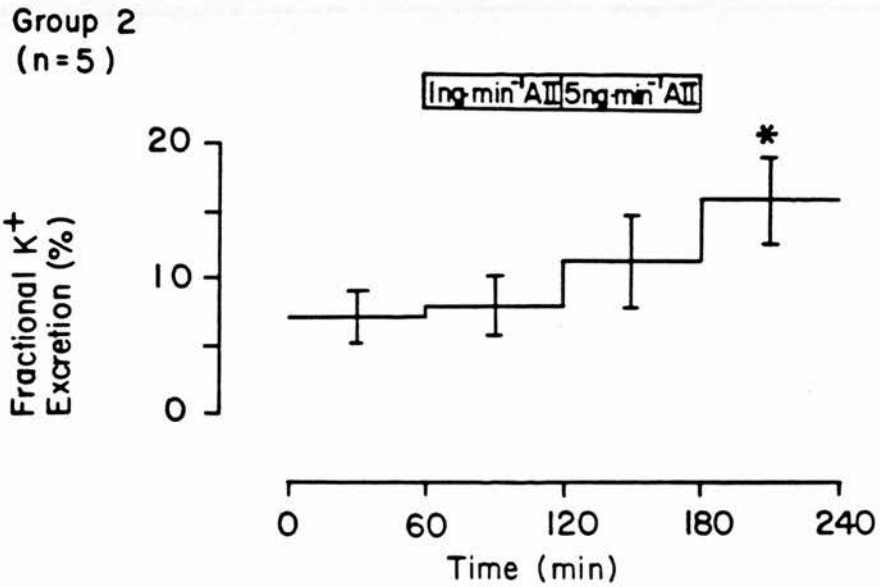


FIG 26 THE EFFECT OF ANGIOTENSIN II ON MEAN POTASSIUM EXCRETION RATE



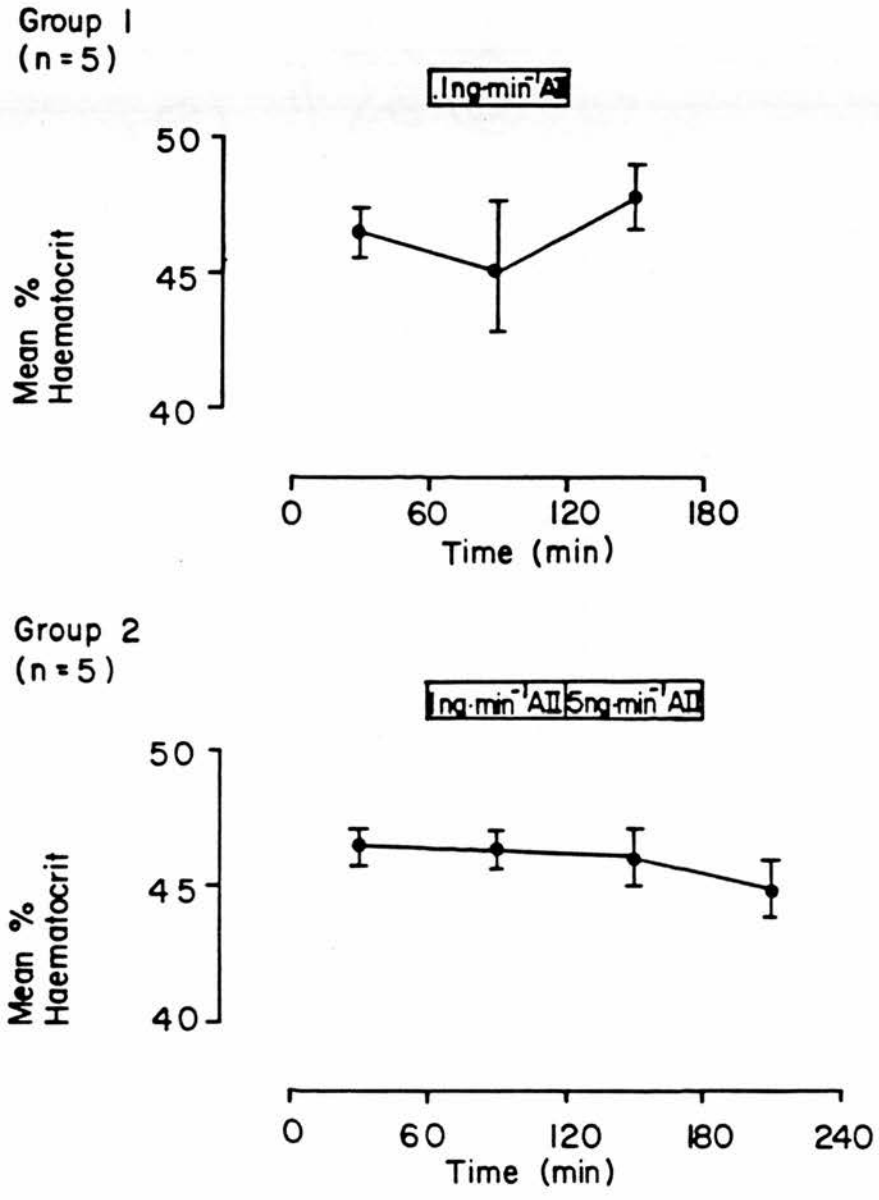
* ,P<0.05 relative to control
† ,P<0.05 relative to Group I

FIG 27 THE EFFECT OF ANGIOTENSIN II ON THE MEAN PERCENTAGE OF FILTERED POTASSIUM WHICH WAS EXCRETED



* , P<0.05 relative to control

FIG 28 THE EFFECT OF ANGIOTENSIN II ON MEAN ARTERIAL HAEMATOCRIT

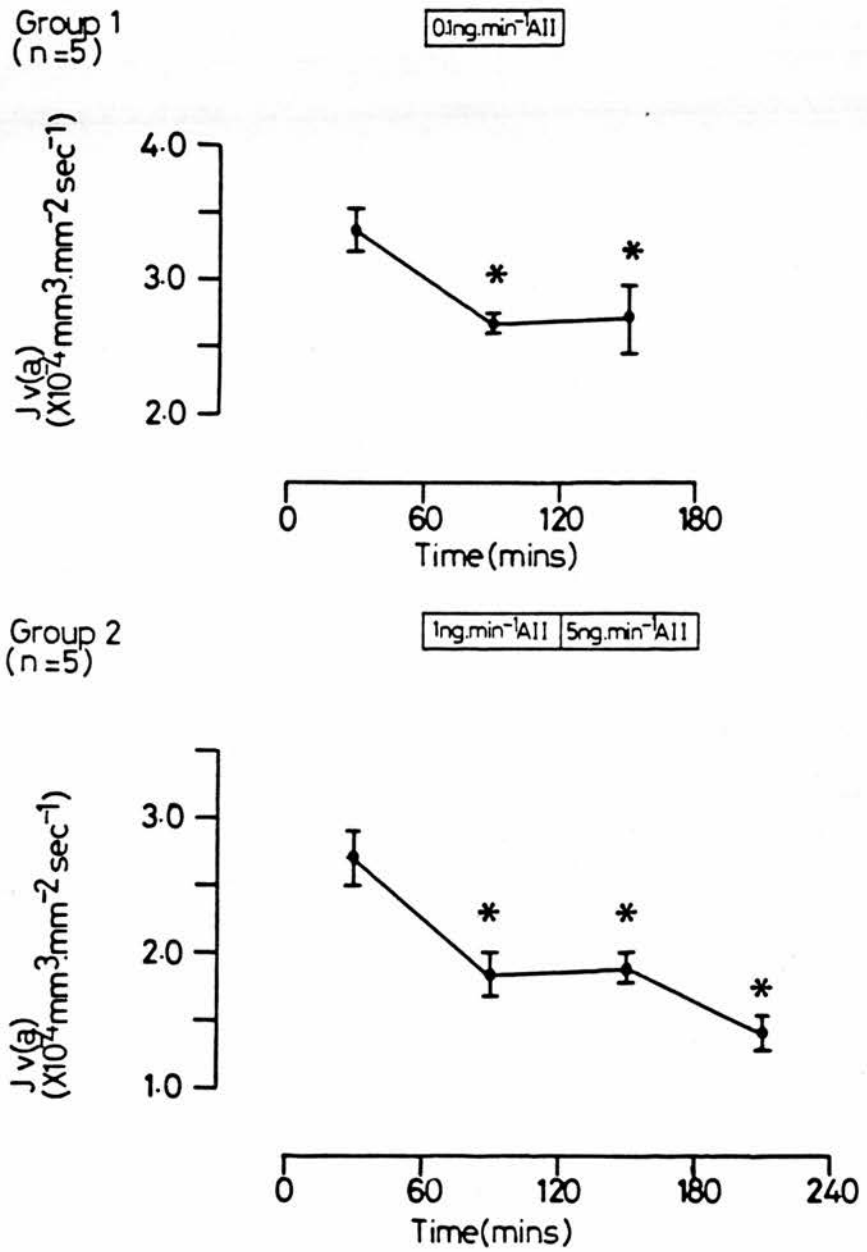


PROXIMAL TUBULAR FLUID REABSORPTION (Jv)

Data obtained for mean Jv are presented in Fig. 29. Infusion of AII at rates of 0.1, 1 and 5 $ng.min^{-1}$ did not increase proximal tubular fluid reabsorption. In group 1, mean Jv during the 0.1 $ng.min^{-1}$ AII infusion period was $2.67 \pm 0.05 \times 10^{-4} mm.^{3}mm.^{-2}sec^{-1}$, a value significantly lower ($P<0.05$) than the initial control value ($3.37 \pm 0.15 \times 10^{-4}$ $mm.^{3}mm.^{-2}.sec^{-1}$), but not significantly different from the recovery period value ($2.70 \pm 0.25 \times 10^{-4}$ $mm.^{3}mm.^{-2}sec^{-1}$). In group 2, the values obtained for mean Jv during the 1 and 5 $ng.min^{-1}$ AII infusion periods were 1.83 ± 0.16 and $1.87 \pm 0.14 \times 10^{-4}$ $mm^{3}.mm^{-2}.sec^{-1}$ respectively. Although these values were significantly lower than the initial control value ($2.71 \pm 0.20 \times 10^{-4}$ $mm^{3}.mm^{-2}.sec^{-1}$, $P<0.05$ in both cases), they were not significantly different from the recovery period value ($1.40 \pm 0.13 \times 10^{-4} mm^{3}.mm^{-2}.sec^{-1}$).

Thus in both groups, although the values obtained for mean Jv during the AII infusion periods were lower than the corresponding control values, they were not significantly different from the corresponding recovery period values. Indeed, in both groups, mean Jv during the recovery period was significantly lower than during the initial control period ($P<0.05$ in both cases).

FIG 29 THE EFFECT OF ANGIOTENSIN II ON MEAN PROXIMAL TUBULAR FLUID REABSORPTION (J v(a))



*, P<0.05 relative to control

DISCUSSION

The present study was designed to examine the renal effects of systemic infusion of low doses of AII in anaesthetised sodium loaded rats. Infusion of AII at rates of 0.1, 1 and 5 $ng.min^{-1}$ did not increase arterial blood pressure or proximal tubular fluid reabsorption or significantly affect urine flow, urinary sodium or potassium excretion, the percentage of filtered sodium reabsorbed or the fractional excretion of potassium. In group 2, glomerular filtration rate during the 5 $ng.min^{-1}$ AII infusion period was significantly lower than the control value but not significantly different from the recovery period value. Similarly, in both groups 1 and 2, although the values obtained for mean Jv during the AII infusion periods were significantly lower than the corresponding control values, they were not significantly different from the corresponding recovery period values. Indeed, both GFR and proximal Jv remained significantly reduced following infusion of AII. Thus, the observed reductions in GFR and Jv during the AII infusion periods may not have been due to infusion of the peptide but may have represented time-dependent decreases in these parameters. Indeed, time-dependent decreases in arterial blood pressure occurred in both groups 1 and 2. Therefore, the observed time-dependent decreases in GFR and proximal Jv may have resulted from the progressive decreases in arterial blood pressure.

The results differ from the general finding that systemic infusion of low (sub-pressor) doses of AII produce an antinatriuresis (Barraclough et al., 1967; Malvin and Vander, 1967; Johnson and Malvin, 1977). In addition, the results showed that infusion of AII at rates of 0.1, 1 and 5 $ng.min^{-1}$ failed to stimulate proximal tubular fluid reabsorption. One possible explanation for the lack of a stimulatory effect on proximal reabsorption or an antinatriuretic effect of low doses of AII is that

the animals used in the present study were insufficiently sodium loaded to supress endogenous AII production. However, in order to ensure a marked sodium loading, animals in group 2 were given 2% NaCl to drink (instead of tap water) for at least five weeks prior to the experiments. Although plasma or intrarenal AII levels were not measured (viable AII radioimmunoassay not available), it is possible that such a degree of sodium loading failed to sufficiently suppress endogenous AII production to allow the infused hormone to elicit an increase in proximal tubular reabsorption and an antinatriuresis. Indeed, Mendelsohn (1979; 1982) reported that in rats, intrarenal AII levels were disassociated from plasma renin activity and kidney renin concentrations during sodium loading, ie, sodium loading depressed plasma renin activity and kidney renin concentration but did not alter intrarenal AII.

However, it is possible that sodium loading did suppress endogenous AII production, but that subsequent induction of Inactin anaesthesia and/or surgical stress may have raised circulating and/or intrarenal AII levels and thus offset the prior effect of sodium loading on endogenous angiotensin production. Indeed, it has been reported that Inactin anaesthesia and surgical stress both increase sympathetic activity and that this increases renin secretion and circulating AII and catecholamine levels (Davis and Freeman, 1976, Fray et al., 1976; Keeton and Campbell, 1980; Tucker et al., 1982; Walker et al., 1983). Therefore, in the present study, anaesthesia and /or surgical stress may have offset the effect of prior sodium loading on endogenous AII production, thereby obscuring any stimulatory effect on proximal reabsorption, or any antinatriuretic effect of infusion of low doses of the peptide. Another possibility is that the doses of AII infused were too low to elicit an increase in proximal reabsorption and/or a pronounced antinatriuresis. Alternatively, systemic AII may not have such actions.

The effects of alterations in sodium intake on renal function have been examined extensively. Hall et al., (1980a and 1980b) increased sodium intake from 5 to 495 mEq per day in normal conscious dogs and observed that urinary sodium excretion, RBF and GFR increased significantly. These changes occurred in the absence of alterations in arterial blood pressure. Other studies have failed to demonstrate an effect of altered sodium intake on RBF, GFR or sodium excretion (Fagard et al., 1978; Luft et al., 1979; Schor et al., 1980; Wong and Zimmerman, 1982). Wong and Zimmerman (1982) reported that arterial pressure, RBF and RVR were unaltered by changes in dietary sodium intake in studies using conscious dogs.

However, Coelho (1974) and Bonvalet et al., (1977) showed that rats fed a high salt diet or salt loaded by saline infusion exhibited significant increases in GFR of both superficial and deep nephrons. In addition, Mendelsohn (1979; 1982) showed that in conscious rats, oral saline loading increased urine flow, urinary sodium concentration and sodium excretion without altering GFR. Sodium loading suppressed plasma renin activity and kidney renin concentration but did not alter intrarenal AII concentration. However, sodium deprivation was found to decrease urinary sodium concentration and increase plasma renin activity and intrarenal AII and renin concentrations. Indeed, intrarenal AII concentration was found to be inversely related to urinary sodium concentration during sodium deprivation (Mendelsohn, 1979;1982). These findings taken together with data from studies using blockers/antagonists of the renin-angiotensin system (Kimbrough et al., 1977; Lohmeier et al. 1977), support the concept that the renin-angiotensin system exerts an important role in the regulation of GFR and sodium excretion during sodium deprivation. However, the finding by Mendelsohn (1979; 1982) that sodium loading did not suppress intrarenal AII levels (ie, that the relationship between sodium balance and intrarenal AII was

non-linear) suggest that other mechanisms are involved in the regulation of sodium excretion during salt loading.

Chronic increases in dietary sodium intake have been found not to significantly alter plasma protein or electrolyte levels despite reducing plasma renin activity and plasma aldosterone concentration (Hall et al., 1980a; 1980b; Haberle and Davis, 1982). However, Haberle and Davis (1982) reported that after about two weeks salt loading rats exhibited significant increases in urine flow, GFR and plasma volume. Thus, dietary sodium loading as well as infusion of isotonic saline or plasma appears to result in extracellular fluid volume expansion.

Extracellular fluid volume expansion due to dietary sodium loading or infusion of isotonic saline or plasma has been shown to increase urinary sodium and water excretion (Landwehr et al., 1967; Bank, 1970; Haberle and Davis, 1982; Knox and Haas, 1982; Knox et al, 1983). The possible mechanisms whereby volume expansion increases urinary sodium and water excretion are discussed in Section 6.

In the present study the two experimental groups of animals were subjected to different degrees of sodium loading. Despite this, the control values for arterial blood pressure, urine flow, urinary sodium excretion, sodium reabsorption and proximal Jv in the two groups were not significantly different. In addition, values for urine flow and sodium excretion obtained during the control periods in both groups were not different from those observed during the control periods in the non-sodium loaded animals (groups 1,2, and 3, Section 4). In this respect, it is not clear why the control urine flow and sodium excretion rates in the sodium loaded animals were not significantly higher than the corresponding values in the non-sodium loaded rats (Section 4). However it is possible that anaesthesia and/or surgical stress may impair the ability of the kidney to excrete sodium.

As a viable AII radioimmunoassay was not available, neither plasma nor intrarenal AII levels were measured in either the present study or the experiments reported in the previous section. However, the present results are consistent with the possibility that in anaesthetised normal (ie, not sodium loaded or depleted) or chronically sodium loaded rats prepared for micropuncture, endogenous angiotensin production is not suppressed and that AII is still produced in amounts almost optimal for maximal stimulation of proximal tubular fluid reabsorption and sodium retention (Barraclough et al., 1967; Mendelsohn, 1979; 1982).

Recently, Radhi et al., (1982) demonstrated the antinatriuretic effect of low doses of AII in anaesthetised surgically stressed rats. However, they carried out their study on acutely volume expanded rats (ie, systemic infusion of isotonic saline at a rate of about 12 ml. hr^{-1}; equivalent to 44-52 $ml.kg^{-1}.hr^{-1}$). Therefore, it is possible that acute saline volume expansion may suppress endogenous AII production in the anaesthetised rat and thus allow the demonstration of a stimulatory effect on proximal tubular fluid reabsorption and an antinatriuretic effect of low doses of exogenous peptide.

SECTION 6

THE RENAL EFFECTS OF ANGIOTENSIN II AND CAPTOPRIL IN ACUTELY VOLUME EXPANDED CHRONICALLY SODIUM LOADED RATS

INTRODUCTION

The experiments reported in Section 5 failed to demonstrate a stimulation of proximal reabsorption or a pronounced antinatriuresis in response to infusion of low doses of AII in anaesthetised sodium loaded rats. As discussed in the previous section, it is possible that the doses of AII infused were too low to elicit an increase in proximal reabsorption and/or a pronounced antinatriuresis. Alternatively, systemic AII may not have such actions. However, another possibility is that endogenous angiotensin production was not sufficiently suppressed to allow the infused hormone to elicit an increase in proximal reabsorption and/or a pronounced antinatriuresis.

Radhi et al., (1982) using an anesthetised rat preparation, reported that systemic infusion of low doses of AII (20 and 50 $ng.kg^{-1}.min^{-1}$) resulted in significant reductions in both urine flow and sodium excretion rate. However, their experiments were performed on acutely volume expanded animals (ie, animals were infused with isotonic saline at a rate of approximately 12 $ml.hr^{-1}$). Therefore, it is possible that acute extracellular fluid volume expansion may sufficiently suppress endogenous angiotensin II production in the anaesthetised surgically stressed rat, to facilitate the demonstration of an antinatriuretic effect of low doses of the peptide.

The experiments reported in this section were designed to determine the effects of intravenous infusion of low (sub-pressor) doses of AII on renal function in the anaesthetised chronically sodium loaded and acutely volume expanded rat. In addition, a second series of experiments were carried out to evaluate the renal effects of systemic infusion of the angiotensin-converting enzyme inhibitor, captopril (SQ 14,225), and of infusion of AII during continuing converting enzyme blockade in the anaesthetised chronically sodium loaded and acutely volume expanded rat.

METHODS

ANIMALS

Experiments were conducted on sodium loaded male Wistar rats (250-310g) fed a standard rat diet. The animals were chronically sodium loaded by allowing them free access to 2% saline for 10-16 days prior to the experiment, and acutely volume expanded by infusing intravenously, 0.9% saline at a rate of 150 $\mu l.min^{-1}$ (9 $ml.hr^{-1}$; 29-36 $ml.kg^{-1}.hr^{-1}$) throughout the duration of the experiment.

SURGICAL PROCEDURE

Rats were anaesthetised and prepared for micropuncture as described in Section 2.

EXPERIMENTAL PROCEDURE

Following placement of the jugular cannulae, a priming injection of 1 ml of 0.9% saline was given to replace fluid loss during surgery and 0.9% saline was infused from both syringes (total rate of 150 $\mu l.min^{-1}$).

Two groups of experiments employing different protocols were performed.

Group 1 - Infusion of 5 and 10 $ng.min^{-1}$ AII (n = 5)

Upon completion of surgery, a priming dose (1 ml of 2% solution) of Inutest was administered through one of the jugular cannulae. A 1% Inutest solution was then infused from one syringe at 75 $\mu l.min^{-1}$ throughout the remainder of the experiment. A period of 90 minutes was then allowed for the attainment of steady state conditions. This stabilisation period was followed by a 60 minute control period during which the animals received infusions only of saline and Inutest. In the two subsequent 60 minute periods, AII was added to the saline syringe

and administered at doses of 5 and 10 ng.min^{-1}. There then followed a final 60 minute recovery period during which only saline and Inutest were infused.

During each 60 minute infusion period, micropuncture procedures were carried out to determine proximal tubular reabsorption (Jv) in randomly selected surface proximal tubules. These procedures are described in Section 2.

Group 2 - Infusion of CEI (Converting Enzyme Inhibitor) and CEI + AII (n = 6)

^{14}C inulin (Amersham) was used as the clearance marker for measurement of GFR. Following surgery, a priming dose (1 ml of 4.6 KBq - 0.124 μCi) of ^{14}C inulin was administered through one of the jugular cannulae. ^{14}C inulin was then infused (75 μl.min^{-1}) continuously during the experiment at the rate of 0.344 KBq.min^{-1} (0.009 μCi.min^{-1}). A 90 minute equilibration period was then allowed for the animals to reach steady state conditions before urine collections were initiated. This equilibration period was followed by a 60 minute control period during which the animals received only saline and ^{14}C inulin. A priming dose (1 mg dissolved in 0.5 ml saline) of converting enzyme inhibitor (CEI) (captopril, SQ14,225) was then administered intravenously through the cannula previously used for infusion of saline alone. CEI was infused through this cannula at the rate of 7.5 μg.min^{-1} throughout the remainder of the experiment. A period of 30 minutes was allowed for attainment of maximum converting enzyme blockade and stabilisation of renal function before initiating a further 60 minute (CEI) period of urine collections. AII was then added to the CEI infusion and administered at a rate of 10 ng.min^{-1} during the third period (CEI + AII).

The effectiveness of converting enzyme inhibition was checked by the

abolition of the pressor response to bolus injections of 50 ng of AI (1 ng.μl^{-1}) given before and after administration of CEI.

In both groups, the total rate of fluid administration remained constant (150 $\mu l.min^{-1}$). Twenty minute urine collections were made during each 60 minute infusion period in both groups of animals. At the mid-point of each 60 minute infusion period, an arterial blood sample (400 µl.) was taken. The cells were separated by centrifugation and the plasma removed. An aliquot of blood was used for estimations of arterial haematocrit. At the end of each experiment, the left kidney was removed, blotted dry and weighed.

RESULTS

MEAN ARTERIAL BLOOD PRESSURE

In group 1, infusion of AII at rates of 5 and 10 ng.min^{-1} did not increase arterial blood pressure. However, during the final recovery period, arterial blood pressure decreased to 101.6 ± 4.8 mmHg, a value significantly lower ($P<0.05$) than during the control period (120.2 ± 2.9 mmHg) (Fig. 30).

Bolus injections of 50 ng of AI (1 ng.µl;n=5) given before administration of CEI resulted in a mean increase in MABP of 27.6 ± 1.0 mmHg while bolus injections of 50 ng of AI given 30 minutes (n=5) and 90 minutes (n=5) after CEI administration produced no measureable increase in MABP. Thus, the dose of CEI used in the present study resulted in the complete abolition of the pressor response to bolus injections of 50 ng of AI.

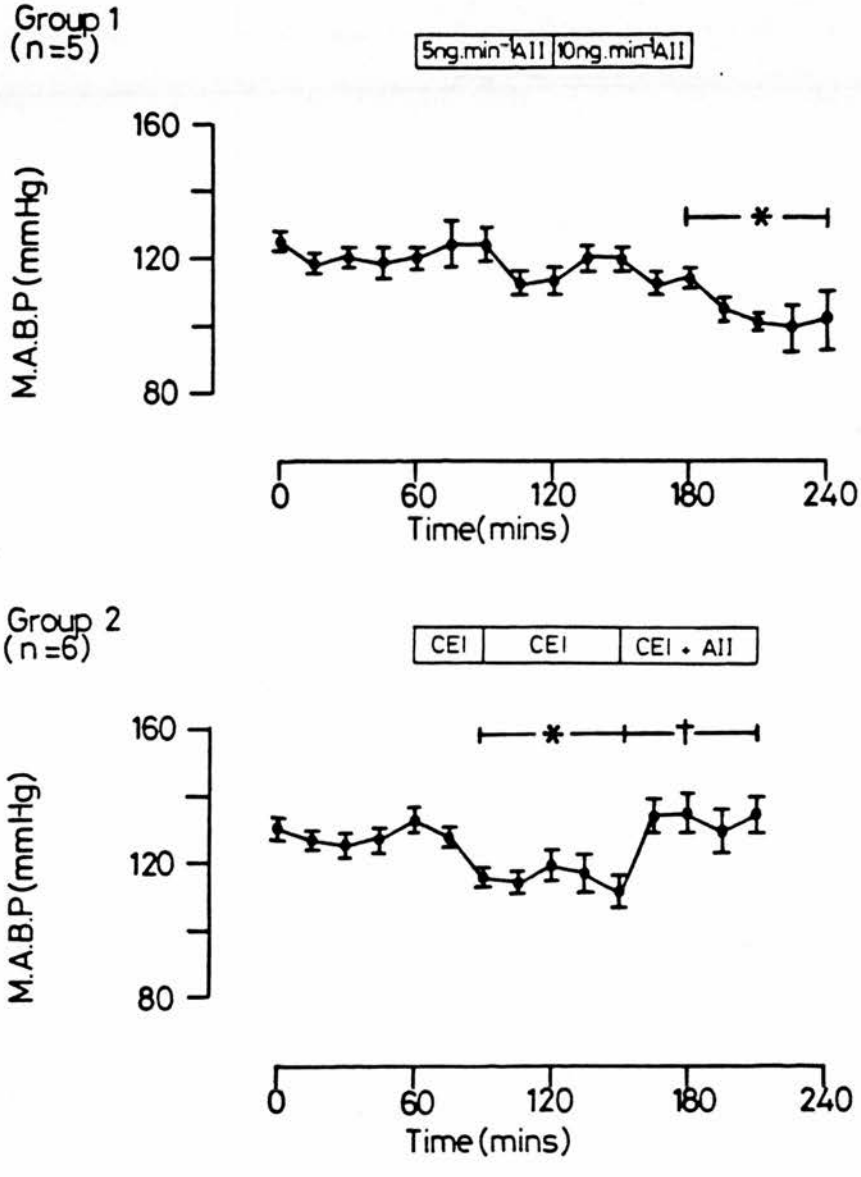
Administration of captopril resulted in a significant decrease in arterial blood pressue from 128.6 ± 3.0 to 115.9 1 2.9 mmHg ($P<0.05$). When AII was added to the CEI infusate, arterial blood pressure increased to 133.3 ± 5.5 mmHg, a value not significantly different from the control value (Fig. 30).

URINE FLOW

The data presented in Fig. 31 show that in group 1, urine flow rate was reduced from 25.39 ± 5.41 µl.min^{-1} during the control period to 17.73 ± 2.97 ($P<0.05$) during infusion of 5 ng.min^{-1} AII and 15.41 ± 3.08 µl.min^{-1} ($P<0.05$) during infusion of 10 ng.min^{-1} AII. During the recovery period, urine flow rate was 18.9 ± 4.69 µl.min^{-1}, a value not significantly different from that observed during the control period or during the 5 and 10 ng.min^{-1} AII infusion periods.

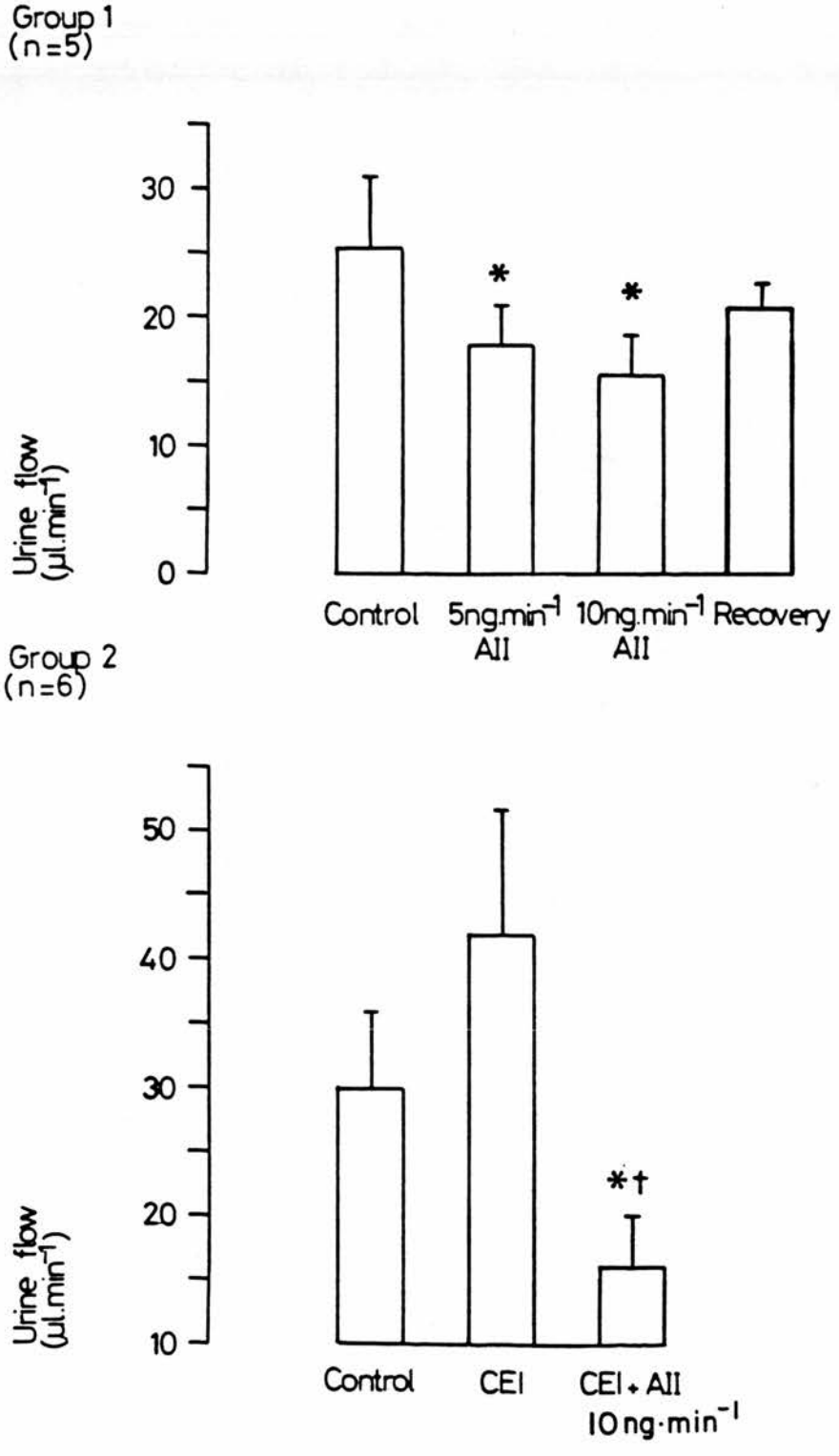
In group 2, urine flow rate increased in five rats and decreased in one rat during the CEI infusion period. Thus, mean urine flow rate

FIG 30 THE EFFECT OF AII, CEI AND CEI + AII ON MEAN ARTERIAL BLOOD PRESSURE



*, $P < 0.05$ relative to control
†, $P < 0.05$ relative to CEI infusion

FIG 31 THE EFFECT OF AII, CEI AND CEI + AII ON MEAN URINE FLOW RATE



*, $P<0.05$ relative to control
†, $P<0.05$ relative to CEI infusion

during the CEI infusion period (41.7 ± 9.79 $\mu l.min^{-1}$) was not significantly different from that during the control period (29.79 ± 6.02 $\mu l.min^{-1}$). Addition of AII to the CEI infusate decreased urine flow rate to 16.01 ± 3.94 $\mu l.min^{-1}$, a value significantly lower than that observed during both the control and CEI infusion periods ($P<0.05$ in both cases).

GLOMERULAR FILTRATION RATE (GFR)

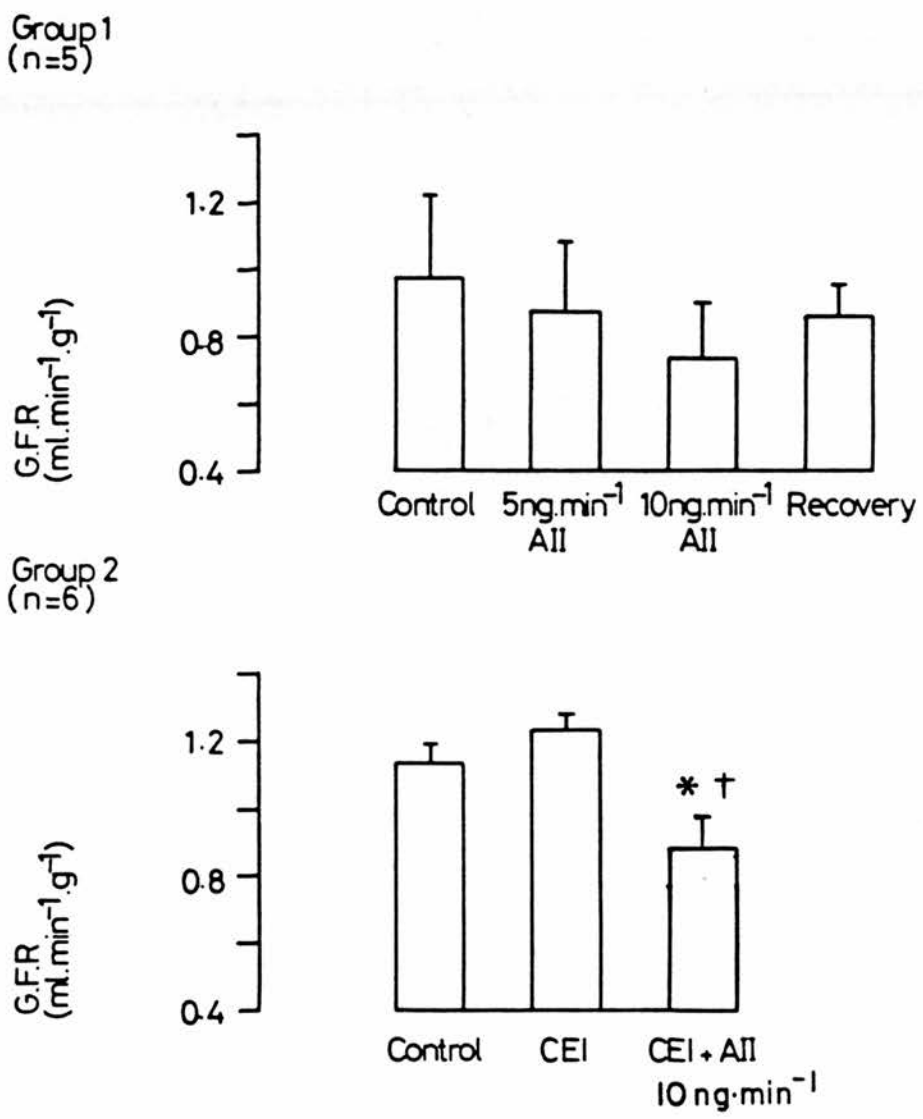
Data obtained for glomerular filtration rate are presented in Fig. 32. Although glomerular filtration rate decreased from 0.97 ± 0.25 $ml.min^{-1}.g^{-1}$ during the control period to 0.87 ± 0.21 and 0.73 ± 0.17 $ml.min^{-1}.g^{-1}$ during the 5 and 10 $ng.min^{-1}$ AII infusion periods, the decreases were not statistically significant. During the recovery period, GFR was 0.86 ± 0.09 $ml.min^{-1}.g^{-1}$, a value not significantly different from the control value.

Infusion of captopril resulted in a small but insignificant increase in GFR from 1.13 ± 0.06 $ml.min^{-1}.g^{-1}$ during the control period to 1.23 ± 0.05 $ml.min^{-1}.g^{-1}$. Addition of AII to the CEI infusion resulted in a decrease in GFR to 0.88 ± 0.08 $ml.min^{-1}.g^{-1}$, a value significantly lower than during both the control and CEI infusion periods ($P<0.05$ in both cases).

SODIUM EXCRETION RATE

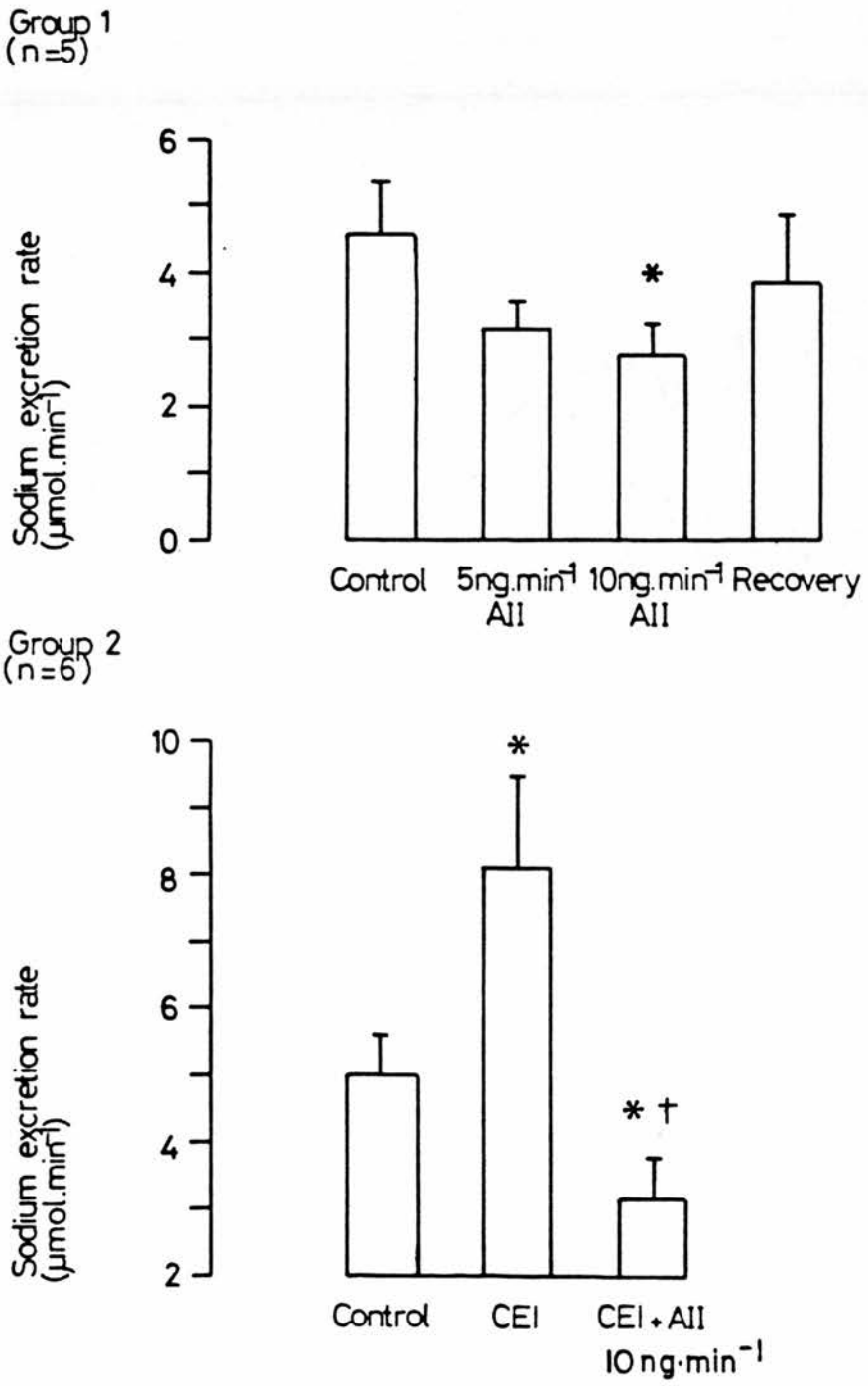
In group 1 (Fig. 33), sodium excretion rate was 3.13 ± 0.40 $\mu mol.min^{-1}$ during the 5 $ng.min^{-1}$ AII infusion period, a value not significantly different from the control value (4.54 ± 0.84 $\mu mol.min^{-1}$). Infusion of AII at 10 $ng.min^{-1}$ reduced sodium excretion rate to 2.73 ± 0.47 $\mu mol.min^{-1}$, a value significantly lower than the control value ($P<0.05$). During the recovery period, sodium excretion was 3.85 ± 0.97 $\mu mol.min^{-1}$, a value not significantly different from the control value.

FIG 32 THE EFFECT OF AII, CEI AND CEI + AII ON MEAN GLOMERULAR FILTRATION RATE



*, $P<0.05$ relative to control
†, $P<0.05$ relative to CEI infusion

FIG 33 THE EFFECT OF AII, CEI AND CEI + AII ON MEAN SODIUM EXCRETION RATE



*, P<0.05 relative to control
†, P<0.05 relative to CEI infusion

Infusion of captopril resulted in a marked increase ($P<0.05$) in sodium excretion rate from 4.99 ± 0.59 $\mu mol.min^{-1}$ during the control period to 8.04 ± 1.43 $\mu mol.min^{-1}$. The natriuresis induced by captopril was completely reversed by the addition of AII to the CEI infusate. During the infusion of CEI and AII, sodium excretion was reduced to 3.07 ± 0.69 $\mu mol.min^{-1}$, a value significantly lower than that observed during both the control and CEI infusion periods (4.99 ± 0.59 and 8.0 ± 1.43 $\mu mol.min^{-1}$ respectively, $P<0.05$ in both cases). These results are shown in Fig. 33.

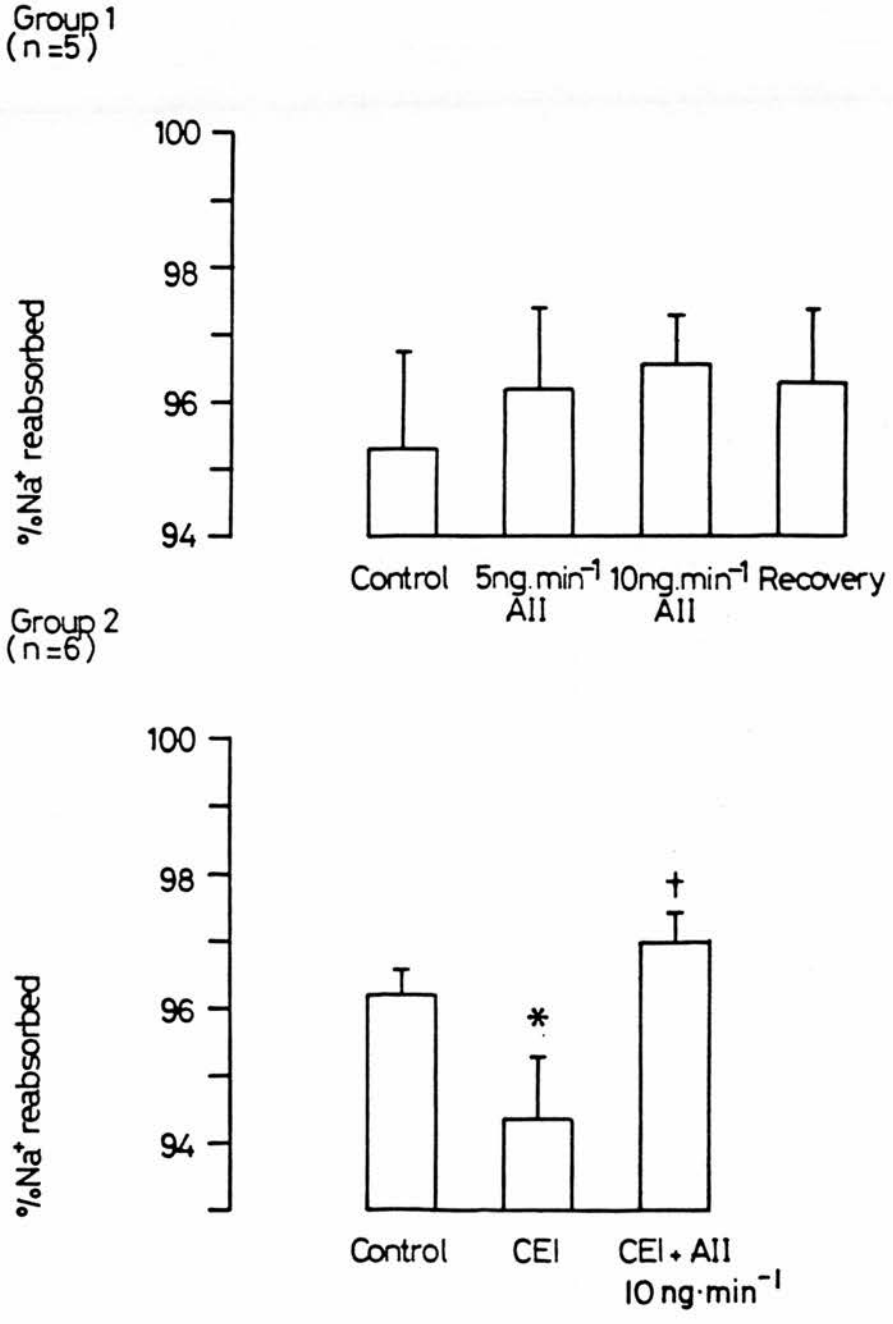
PERCENTAGE OF FILTERED SODIUM WHICH WAS REABSORBED

The results presented in Fig. 34 show that there was no significant change in this parameter during the 5 and 10 $ng.min^{-1}$ AII infusion experiments. However, sodium reabsorption decreased from 96.18 ± 0.39 to 94.34 ± 0.93% of filtered load ($P<0.05$) during CEI infusion. Infusion of AII during converting enzyme blockade increased fractional sodium reabsorption to 96.96 ± 0.44% of filtered load, a value significantly higher ($P<0.05$) than that observed during the CEI infusion period (Fig. 34).

ABSOLUTE SODIUM REABSORPTION (Fig. 35)

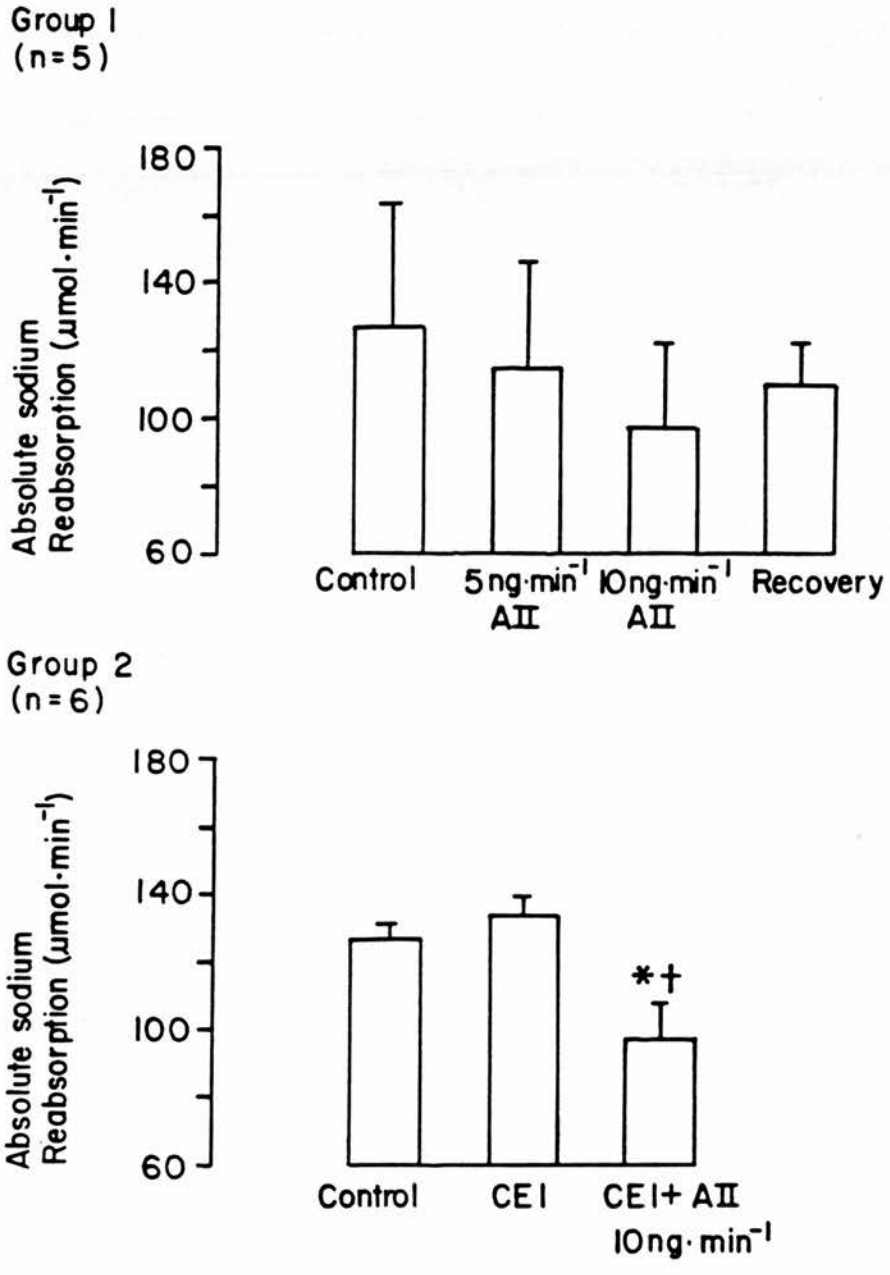
Absolute sodium reabsorption did not vary significantly during the 5 and 10 $ng.min^{-1}$ AII infusion experiments. In group 2, infusion of captopril resulted in a small but statistically insignificant increase in absolute sodium reabsorption from 125.61 ± 5.21 to 133.48 ± 5.31 $\mu mol.min^{-1}$. Addition of AII to the CEI infusate reduced absolute sodium reabsorption to 97.08 ± 10.70 $\mu mol.min^{-1}$, a value significantly lower than that observed during both the control and CEI infusion periods ($P<0.05$ in both cases).

FIG 34 THE EFFECT OF AII, CEI AND CEI + AII ON THE MEAN PERCENTAGE OF FILTERED SODIUM WHICH WAS REABSORBED



*, P<0.05 relative to control
†, P<0.05 relative to CEI infusion

FIG 35 THE EFFECT OF AII, CEI AND CEI+AII ON MEAN ABSOLUTE SODIUM REABSORPTION



* ,P< 0.05 relative to control

† ,P< 0.05 relative to CEI infusion

POTASSIUM EXCRETION RATE

The data obtained for this parameter are presented in Fig. 36. In group 1, the potassium excretion rate decreased from 0.50 ± 0.10 μmol. min^{-1} during the control period to 0.42 ± 0.06 ($P<0.05$) during infusion of 5 ng.min^{-1} AII and 0.41 ± 0.06 μmol.min^{-1} ($P<0.05$) during infusion of 10 ng.min^{-1} AII. During the recovery period, mean potassium excretion rate was 0.50 ± 0.06 μmol.min^{-1}, a value not significantly different from the control value.

In group 2, the potassium excretion rate was not significantly altered during the CEI infusion. However, addition of AII to the CEI infusate resulted in a decrease in the absolute rate of potassium excretion to 0.44 ± 0.10 μmol.min^{-1}, a value significantly lower than that during both the control and CEI infusion periods (0.69 ± 0.17 and 0.74 ± 0.15 μmol.min^{-1}respectively, $P<0.05$ in both cases).

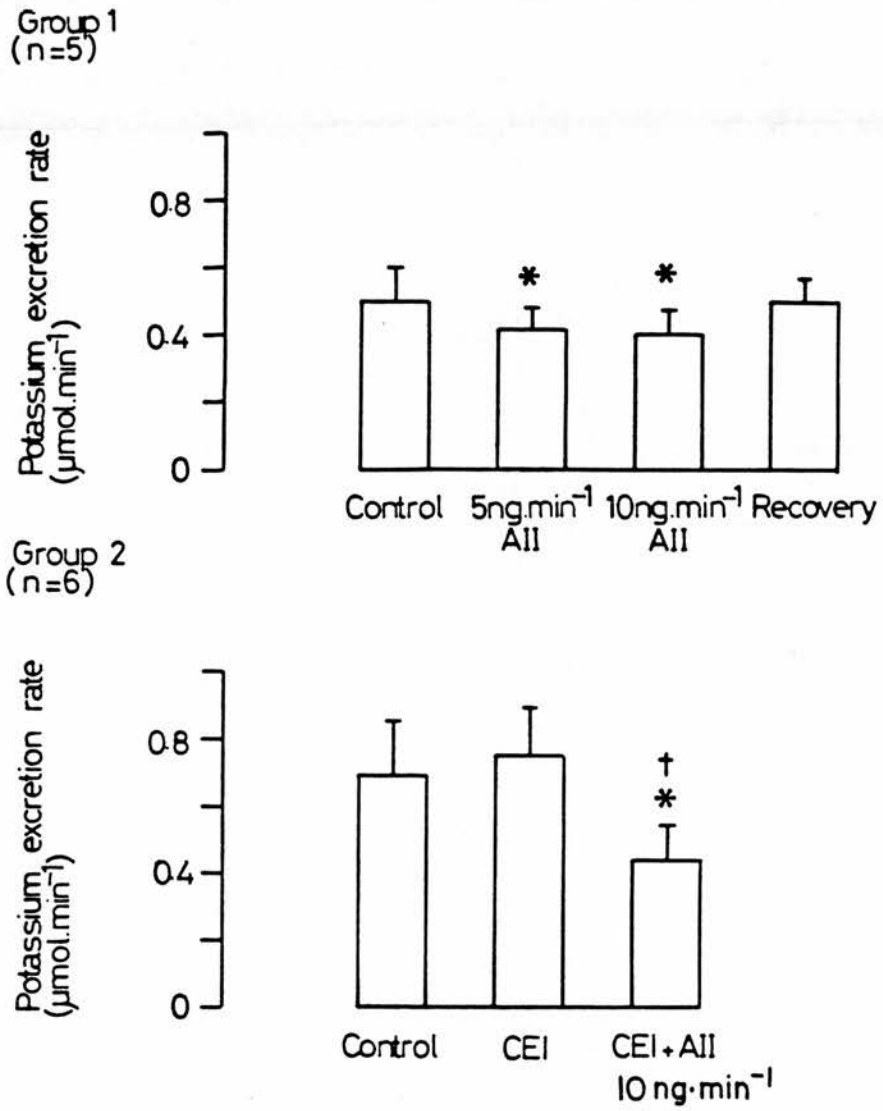
PERCENTAGE OF FILTERED POTASSIUM WHICH WAS EXCRETED

As illustrated in Fig. 37 no significant change in fractional potassium excretion was observed in group 1. In group 2, fractional potassium excretion during the CEI infusion period was 19.78 ± 4.08% of the filtered load, a value not significantly different from the control value (19.38 ± 4.22%). During the CEI + AII infusion period, fractional potassium excretion was 16.14 ± 3.45% of the filtered load, a value significantly lower than that observed during the CEI infusion period ($p<0.05$).

HAEMATOCRIT

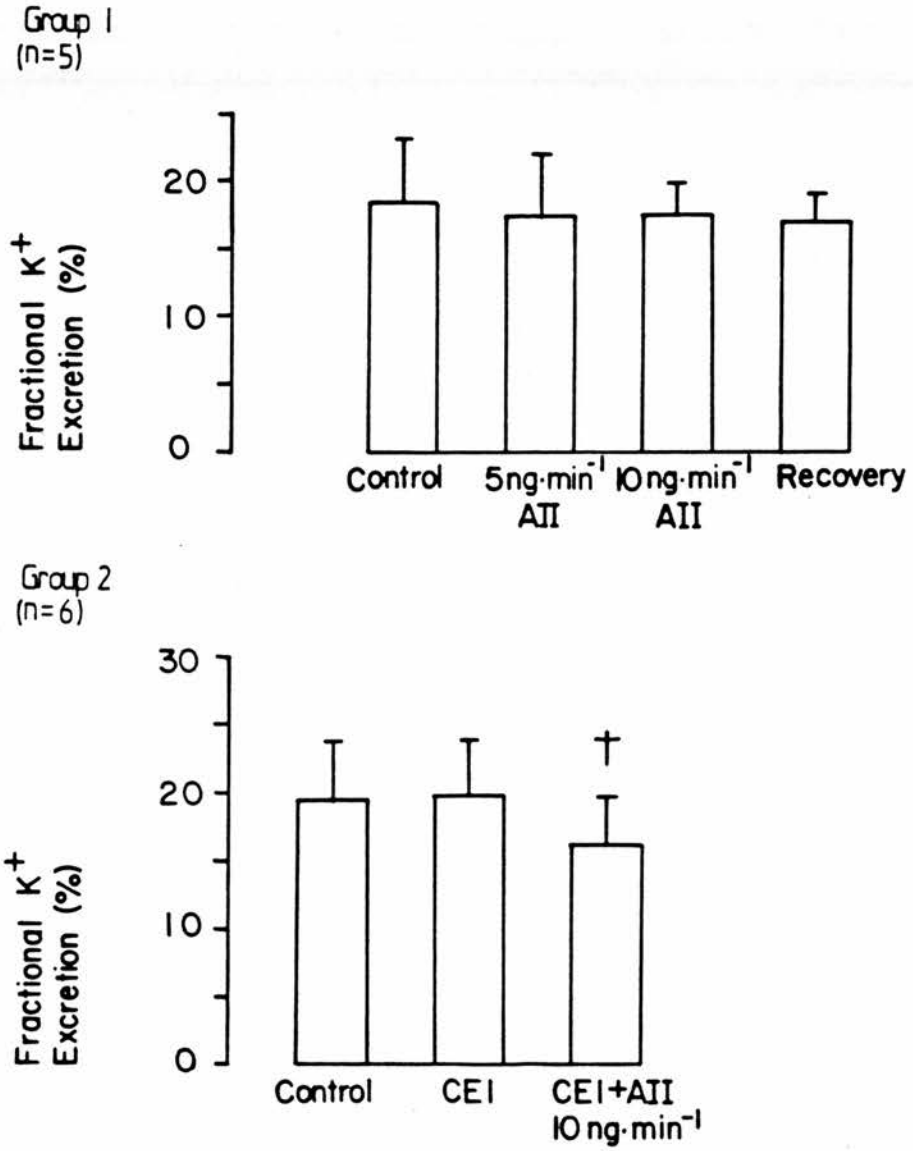
As shown in Fig. 38 arterial haematocrit determined during the 5 ng.min^{-1} AII infusion period (44.3 ± 0.7%) did not differ significantly from the control value (44.7 ± 0.8%). However, haematocrit decreased significantly to 41.7 ± 1.4% ($P<0.05$) during the 10 ng.min^{-1} AII infusion period and to 39.2 ± 1.1% ($P<0.05$) during the recovery period.

FIG 36 THE EFFECT OF AII, CEI AND CEI + AII ON MEAN POTASSIUM EXCRETION RATE



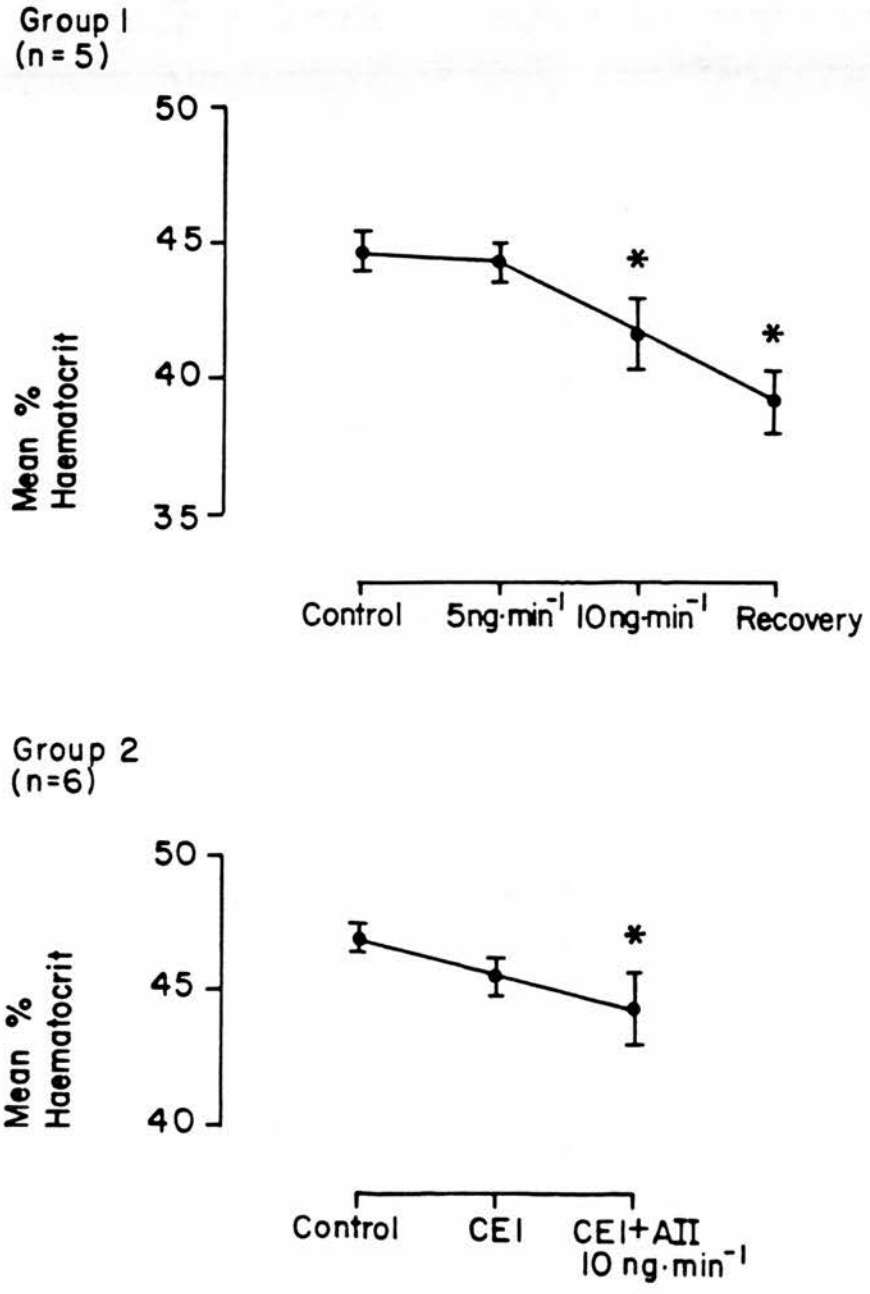
*, $P<0.05$ relative to control
†, $P<0.05$ relative to CEI infusion

FIG 37 THE EFFECT OF AII, CEI AND CEI+AII ON THE MEAN PERCENTAGE OF FILTERED POTASSIUM WHICH WAS EXCRETED



†, P<0.05 relative to CEI infusion

FIG 38 THE EFFECT OF AII, CEI AND CEI+ AII ON MEAN ARTERIAL HAEMATOCRIT



*, $P<0.05$ relative to control

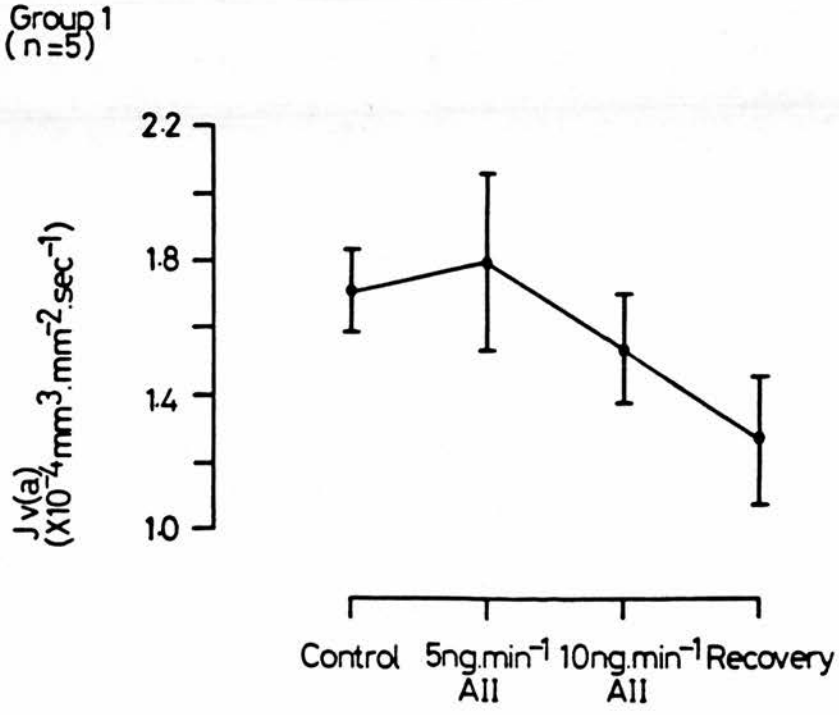
In group 2, mean arterial haematocrit during the CEI infusion period was 45.6 ± 0.7%, a value not significantly different from the control value (46.9 ± 0.5%). During the CEI + AII infusion period, arterial haematocrit was 44.3 ± 1.3%, a value significantly lower than that observed during the control period ($P<0.05$).

PROXIMAL TUBULAR FLUID REABSORPTION

In group 1, mean Jv during the control period was 1.71 ± 0.12 x 10^{-4} $mm^3.mm^{-2}.sec^{-1}$, a value somewhat lower than the corresponding values presented in Sections 3, 4 and 5. As shown in Fig. 39, there were no significant changes in mean Jv during the 5 and 10 $ng.min^{-1}$ AII infusion experiments.

Due to technical difficulties, no micropuncture data were obtained from the CEI + AII infusion experiments (group 2).

FIG 39 THE EFFECT OF AII ON MEAN PROXIMAL TUBULAR FLUID REABSORPTION (Jv(a))



DISCUSSION

The present study showed that in the acutely volume expanded, chronically sodium loaded rat, systemic infusion of AII at a rate of 10 $ng.min^{-1}$ (40 $ng.kg^{-1}.min^{-1}$) significantly reduced urine flow and urinary sodium and potassium excretion without significantly altering GFR or sodium reabsorption. Moreover, the reductions in urine flow and sodium excretion observed during the 10 $ng.min^{-1}$ AII infusion period (Fig. 31-36) occurred in the absence of significant alterations in the rate of proximal tubular fluid reabsorption. Thus, as for the results from Section 5, the data obtained from the 5 and 10 $ng.min^{-1}$ AII experiments (group 1) do not support the concept that the mechanism whereby systemic AII reduces urine flow and urinary sodium excretion involves a direct stimulatory effect of the peptide on proximal tubular fluid reabsorption. However, it is possible that the peptide may have stimulated tubular reabsorption by more distal segments of the nephron.

As discussed in Sections 4 and 5, the anaesthetised rat may produce amounts of AII that are optimal for maximal stimulation of proximal tubular reabsorption, and that it may be necessary to suppress endogenous angiotensin production in order to observe an increase in proximal reabsorption and a pronounced antinatriuresis in response to infusion of exogenous hormone. In the present study, rats were chronically sodium loaded for 10-14 days prior to the experiments and received a high rate of intravenous saline infusion (approximately 9 $ml.hr^{-1}$) throughout the experiment. However, in group 1, infusion of AII at a rate of 10 $ng.min^{-1}$ did not significantly alter the rate of proximal tubular fluid reabsorption, despite reducing both urine flow and urinary sodium excretion. Thus, it is possible that endogenous angiotensin production was not sufficiently suppressed to allow infused hormone to elicit an increase in proximal tubular fluid reabsorption.

Alternatively, systemic AII may not have a stimulatory action on proximal tubular fluid reabsorption. However, another possibility is that endogenous AII production was suppressed and that the infused hormone did stimulate proximal tubular sodium reabsorption. This increase may have been masked by an inhibitory effect of acute saline loading on proximal tubular fluid reabsorption.

Since a viable AII assay was not available, kidney and plasma AII concentrations were not determined in the present study. Thus, it is not known what plasma or kidney AII concentrations were, or to what levels they were raised by the doses of AII infused in the present study.

Extracellular fluid volume expansion due to dietary sodium loading or infusion of isotonic saline results in a decrease in renal sodium and water reabsorption and an increase in sodium and water excretion (De Wardener et al., 1961; Earley and Friedler, 1964; Landwehr et al., 1967; Bank, 1970; Knox and Haas, 1982). Although volume expansion has been shown to decrease renal vascular resistance and increase renal blood flow (Barger et al., 1961; Earley and Friedler, 1965) there is no convincing evidence that an increase in renal blood flow per se is responsible for the natriuresis. Indeed, Hartupee et al., (1982) demonstrated that renal arterial infusion of acetylcholine increased renal blood flow, renal interstitial pressure and urinary sodium excretion. However, when the increase in interstitial pressure was prevented by decapsulation of the kidney and partial aortic clamping, the acetylcholine-induced vasodilation did not result in an increase in urinary sodium excretion.

The natriuresis which follows volume expansion has been shown to be independent of changes in glomerular filtration rate and circulating adrenocortical hormone and ADH levels (De Wardener et al., 1961; Levinsky

and Lalone, 1963; Rector et al., 1964; Earley and Friedler, 1965). In addition, although some studies have shown that volume expansion is associated with the redistribution of renal blood flow or glomerular filtration rate (Munck et al., 1970; Blantz et al., 1971; de Rouffignac and Bonvalet, 1970; Jamison and Lacy, 1971) others have shown no redistribution of blood flow and filtrate during volume expansion (Carriere et al., 1972; Bruns et al., 1974; Coelho, 1974; Kinney and Di Scala, 1974).

It is well established that chronic and acute volume expansion result in a decrease in sodium and water reabsorption by the proximal tubule, and that the increase in urinary sodium and water excretion observed during volume expansion is due largely to the decreased rate of tubular reabsorption by this segment of the nephron (Giebisch et al., 1964; Cortney et al., 1965; Dirks et al., 1965; Watson,1966; Landwehr et al., 1967; Rector et al., 1967; Bank et al., 1969; Bank, 1970; Knox and Haas, 1982). Although the precise mechanism for this decrease in proximal reabsorption is unclear, it has been suggested that it is mediated primarily by altered intrarenal physical factors, especially peritubular capillary and interstitial hydrostatic pressure (Martino and Earley, 1967; Bank et al., 1969; Earley and Schrier, 1973; Fitzgibbons et al., 1974; Osgood et al., 1977; Marchand, 1978; Knox and Haas, 1982).

There is evidence that reduced sympathetic nervous system activity (ie, reduced efferent renal sympathetic nerve activity and reduced circulating catecholamine levels) or increased secretion of a natriuretic hormone may also be involved in mediating the decrease in proximal reabsorption associated with volume expansion (De Wardener, 1978; Knox et al., 1983; Di Bona 1982; Knox and Haas, 1982). However, the role for such factors remains unclear. Indeed, it has been demonstrated that reducing renal artery pressure prior to volume expansion prevents the decrease in proximal tubular reabsorption and the increases in interstitial hydrostatic pressure and sodium excretion (Bank et al., 1969;

Fitzgibbons et al., 1974; Osgood et al., 1977; Marchand, 1978). These results provide suggestive evidence against an important role for a reduction in sympathetic nervous system activity or a circulating hormone as mediators of the decrease in proximal reabsorption and the natriuresis associated with volume expansion.

In the present study, proximal tubular fluid reabsorption was estimated using the shrinking droplet micropuncture technique. The shrinking droplet involves net volume reabsorption and is subject to altered peritubular physical forces (Sato, 1975). Thus, although AII might, in the present study, have stimulated proximal tubular sodium reabsorption, the finding that systemic AII did not significantly alter proximal tubular fluid reabsorption may have been due to an inhibitory effect of saline volume expansion on tubular fluid reabsorption. Therefore, use of methods for determining proximal tubular sodium reabsorption, as opposed to tubular fluid reabsorption, such as the stationary droplet microperfusion technique, may, in the future, resolve this. The stationary droplet microperfusion technique separates transepithelial sodium and water movement from transendothelial fluid uptake and allows the measurement of active sodium transport. Unlike the shrinking droplet, which involves net volume flux and is subject to inhibition by increased peritubular capillary hydrostatic pressure, the stationary droplet is probably not affected by changes in peritubular capillary hydrostatic pressure.

Infusion of the converting enzyme inhibitor, captopril (SQ 14,225), resulted in small but insignificant increases in glomerular filtration rate and urine flow and significant increases in sodium and potassium excretion and a marked decrease in the fraction of filtered sodium which was reabsorbed (Fig. 31-35). These responses occurred despite a significant decrease in arterial blood pressure. These results are in good agreement with previous reports concerning the characteristics of

converting enzyme inhibition in rats (Thurston and Swales, 1978; Ploth et al., 1979; Huang et al., 1981; Harris and Munro, 1984). Infusion of AII (10 $ng.min^{-1}$) during continuing converting enzyme blockade restored arterial blood pressure and fractional sodium reabsorption to control levels and produced significant reductions in glomerular filtration rate, urine flow and urinary sodium and potassium excretion (Fig. 30-36). The reduction in absolute sodium reabsorption that occurred during the CEI + AII infusion period may be explained on the basis of a reduced GFR and filtered sodium load and may not be due to an AII mediated reduction in tubular reabsorption. Indeed, addition of AII to the CEI infusate significantly increased the percentage of filtered sodium which was absorbed.

Thus, during continuous blockade of endogenous AII production, infusion of the peptide at a rate of 10 $ng.min^{-1}$ resulted in a significant antidiuresis and antinatriuresis. These changes may have been effected entirely by the concomitant reduction in GFR. However, it is also possible that AII exerted a stimulatory action on tubular reabsorption and that this may have contributed to the decrease in sodium and water excretion. Such an action is indicated since fractional sodium reabsorption increased significantly during the CEI and AII infusion period.

As AII has been shown to stimulate aldosterone secretion (Laragh and Sealey, 1973; Hall et al., 1979b; MaCaa, 1982), it is possible that the effects of captopril and AII could be mediated by changes in aldosterone secretion. Indeed, it has been demonstrated that infusion of captopril in conscious dogs reduces plasma aldosterone concentration and that infusion of AII during continuing converting enzyme blockade restores plasma aldosterone to control levels (Hall et al., 1979b; MaCaa, 1982). However, infusion of aldosterone during captopril infusion has been found not to significantly alter renal function from the values

observed during captopril infusion alone (Hall et al., 1979b; MaCaa, 1982). In the present study, the natriuresis observed during captopril administration and the decreases in sodium excretion observed during infusion of AII in both groups 1 and 2 were rapid in onset and readily reversible, while the sodium retaining action of aldosterone is relatively slow and requires a latent period of 45 to 120 min before onset (Barger et al., 1958; Ganong and Mulrow, 1958; Ludens and Fanestil, 1976). Similarly, aldosterone administration generally promotes sodium retention and potassium loss, while in the present study, infusion of AII decreased potassium excretion to below control levels in both groups 1 and 2. Together, these results indicate that the effects of captopril and AII observed in the present study were not primarily mediated by changes in aldosterone secretion.

There is a substantial body of evidence which favours the hypothesis that the effects of converting enzyme inhibitors upon renal function are due to the decreased production of AII rather than to the raised intrarenal concentrations of bradykinin and prostaglandins that also accompany inhibition of kininase II (Marks et al., 1980). This evidence has been reviewed recently by Hollenberg (1982) and is further supported by the findings that the renal responses to captopril or teprotide were reversed by infusion of AII (Hall et al., 1979b; MaCaa, 1982; Carmines et al., 1983; Harris and Munro, 1984). In addition, both converting enzyme inhibition and infusion of the AII receptor antagonist, saralasin, have been found to produce similar changes in renal function, characterised by vasodilation, increased glomerular filtration rate, diuresis and natriuresis, despite an accompanying reduction in systemic arterial pressure (Ploth et al., 1979; Ploth and Roy, 1982a). Furthermore, it has been demonstrated that inhibition of prostaglandin synthesis with indomethacin does not block the ability of captopril to increase renal blood flow or glomerular filtration rate

(Wong and Zimmerman, 1980; De Forrest, et al., 1982). The present findings that infusion of AII completely reversed the renal actions of captopril provide further support for the hypothesis that the hypotensive and natriuretic actions of converting enzyme inhibitors are due to inhibition of AII formation and not to increased circulating and renal concentrations of kinins and prostaglandins.

Mendelsohn (1979, 1982) reported that after oral sodium loading, rats had suppressed plasma renin activity and kidney renin concentration but unchanged intrarenal AII levels. Therefore in the present study despite chronic sodium loading and acute saline volume expansion, it is possible that intrarenal AII levels were not suppressed. Indeed, infusion of captopril resulted in small but insignificant increases in GFR and urine flow and a significant increase in sodium excretion. In addition, infusion of AII (10 $ng.min^{-1}$) during continuing converting enzyme blockade resulted in a significant antidiuresis and antinatriuresis. These results are consistent with the concept that under conditions of chronic sodium loading and acute volume expansion, endogenous AII production is not completely suppressed and that endogenous AII is still exerting sodium retaining actions on the kidney (Barraclough et al., 1967; Mendelsohn, 1979; 1982).

If intrarenal AII levels are not suppressed during chronic sodium loading and/or acute volume expansion, then presumably factors other than the altered status of the renin-angiotensin system are involved in the regulation of sodium excretion under such conditions.

In summary, in the chronically sodium loaded rat receiving a high rate of intravenous saline infusion, systemic infusion of sub-pressor doses of AII resulted in significant decreases in urine flow and urinary sodium and potassium excretion. These changes occurred in the absence of significant alterations in either GFR or proximal tubular fluid reabsorption. Under the same conditions, infusion of the converting

enzyme inhibitor, captopril, resulted in increases in urinary sodium and potassium excretion and a reduction in the percentage of filtered sodium reabsorbed. These changes occurred despite a significant decrease in systemic arterial blood pressure. Infusion of AII (10 $ng.min^{-1}$) during converting enzyme blockade reversed the renal responses to captopril and caused significant decreases in GFR, urine flow and sodium excretion and an increase in fractional sodium reabsorption. These results support the concept that under conditions of chronic sodium loading and acute volume expansion, AII production is not completely suppressed and that endogenous AII still exerts sodium retaining actions on the kidney. Although the results are consistent with the notion that AII plays a role in the regulation of renal function via actions on both the renal vasculature and the renal tubules, the present study provides no evidence in support of the concept that systemic AII elicits a decrease in urine flow and sodium excretion via a stimulation of the rate of proximal tubular fluid reabsorption.

CONCLUSIONS

As discussed in Section 1, it is clear that all the components necessary for the generation of the active angiotensin peptides are present in the kidney, that various structures within the kidney possess receptors for angiotensin and that angiotensin exerts multiple actions in the regulation of renal function.

The experiments described in this thesis were designed to investigate the effects of exogenous and endogenous angiotensin on renal function in the rat. The effects of alterations in circulating angiotensin levels and the actions of angiotensin as circulating hormone were assessed by evaluating the responses to systemic infusions of the peptide. It was shown that a high dose of angiotensin resulted in a marked increase in both urine flow and urinary sodium excretion. It appeared that the natriuretic response was dependent upon the increase in renal perfusion pressure. Although it was not possible to determine the mechanism whereby an angiotensin induced increase in renal perfusion pressure resulted in a diuresis and natriuresis it was suggested that it involves an increase in GFR and/or a decrease in tubular reabsorption secondary to increased capillary and/or interstital hydrostatic pressure.

Infusion of small (sub-pressor) doses of angiotensin resulted in a subtle antidiuresis and antinatriuresis when the effects of the peptide were compared to the effects of infusion of saline alone. In addition, infusion of AII at a rate of 10 $ng.min^{-1}$ was found to significantly reduce glomerular filtration rate. These effects of the peptide occurred in the absence of an increase in proximal tubular reabsorption. Indeed, the effects of infusion of sub-pressor doses of AII on proximal tubular reabsorption were not signficantly different to the effects of saline infusion alone. Infusion of small (sub-pressor) doses of AII in acutely

volume expanded, sodium loaded rats, resulted in significant reductions in urine flow and urinary sodium excretion. These changes occurred in the absence of significant alterations in GFR or proximal tubular fluid reabsorption. Together, these results were interpreted as being consistent with the concept that in it's normal state, the rat produces almost optimal antinatriuretic amounts of angiotensin in order to maintain sodium homeostasis, and that it is necessary to suppress endogenous angiotensin production in order to demonstrate a pronounced antinatriuretic effect of the infused hormone. In addition, the results provided no evidence in support of the concept that the mechanism whereby systemic AII reduces urine flow and sodium excretion involves a direct stimulatory effect of the peptide on proximal tubular fluid reabsorption.

Infusion of the converting enzyme inhibitor captopril, in acutely volume expanded, sodium loaded rats resulted in small but statistically insignificant increases in urine flow and GFR, a significant increase in sodium excretion and a decrease in the percentage of filtered sodium that was reabsorbed. Infusion of AII (10 $ng.min^{-1}$) during continuing converting enzyme blockade resulted in significant decreases in urine flow, urinary sodium excretion and GFR and a significant increase in the percentage of filtered sodium that was reabsorbed. These findings were interpreted as indicating that even under conditions of chronic sodium loading and acute volume expansion, endogenous angiotensin still exerts sodium retaining actions on the kidney. In addition, these results were consistent with the possibility that under conditions of converting enzyme inhibition, systemic AII reduces urine flow and urinary sodium excretion via actions on both the renal vasculature and renal tubules.

The data reported in this thesis are consistent with the concept that angiotensin is involved in the regulation of glomerular filtration rate, tubular reabsorption and urinary sodium and water excretion. However, further studies are required to determine the precise site and mechanism of action of angiotensin in the regulation of renal function.

In addition, studies are required to evaluate the quantitative contribution of both intrarenally formed and circulating angiotensin in the regulation of renal function.

BIBLIOGRAPHY

Abrash, L., Walter, R. and Marks, N. (1971). Inactivation studies of angiotension II by purified enzymes. Experientia 27 , 1352-1353.

Ackerly, J.A. and Peach, M.J. (1975). Metabolism of angiotensins in the rabbit myocardium (abstr.) Pharmacologist 17, 235.

Aguilera, G. and Catt, K.J. (1978). Regulation of aldosterone secretion by the renin-angiotensin system during sodium restriction in the rat. Proc. Natl. Acad. Sci. USA. 74, 4057-4061.

Aiken, J.W. and Vane, J.R. (1970). Renin-angiotensin system: Inhibition of converting enzyme in isolated tissues. Nature 228, 30-34.

Alexander, J.C. and Meyer, J.H. (1981). Comparision of Captopril with placebo in the treatment of essential hypertension. In: Angiotensin Converting Inhibitors. Mechanisms of Action and Clinical Implication, edited by Horovitz, Z.P. Baltimore, Urban and Schwarzenberg, pp. 379-392.

Al-Merani, S.A.M.A., Brooks, D.P., Chapman, B.J. and Munday, K.A. (1978). The half-lives of angiotensin II, angiotensin II-amide, angiotensin III, 1-sar-8-ala-angiotensin II and renin in the circulating system of the rat. J. Physiol. 278, 471-490.

Alpern, R.J. and Rector, F.C.,Jr. (1983). Effect of volume expansion (VE) on late proximal tubular bicarbonate absorption ($J_{HCO_3^-}$) in the in vivo microperfused rat proximal convoluted tubule. Kidney Int. 23, 288.

Amorim, D.S., Ferreira, S.H., Manco, J.C., Tanaka, A., Sader, A.A. and Cardoso, S. (1967). Potentiation of circulatory effects of bradykinin by a factor contained in the BOTHROPS jararaca venom. Cardiologia 50, 23-32.

Anderson, R.C., Herbert, P.N. and Mulrow, P.J. (1968). A comparison of the properties of renin obtained from the kidney and uterus of the rabbit. Am. J. Physiol. 215, 774-778.

Anderson R.F., Taher, M.S., Cronic, R.E., McDonald, K.M. and Schrier, R.W. (1975). Effect of β-adrenergic blockade and inhibitors of angiotensin II and prostaglandins on renal autoregulation. Am. J. Physiol. 179, 731-736.

Andreoli, T.E. and Schafer, J.A. (1978). Volume absorption in the pars recta. III. Luminal hypotonicity as a driving force for isotonic volume absorption. Am. J. Physiol. 234, F349-F355.

Andreoli, T.E. and Schafer, J.A. (1979a). Effective luminal hypotonicity: the driving force for isotonic proximal tubular fluid absorption. Am. J. Physiol. 236, F89-F96.

Andreoli, T.E. and Schafer, J.A. (1979b). External solution driving forces for isotonic fluid absorption in proximal tubules. Fed. Proc. 38, 154-160.

Andreoli, T.E., Schafer, J.A. and Troutman, S.L. (1978). Perfusion rate-dependence of transepithelial osmosis in isolated proximal convoluted tubules: estimation of the hydraulic conductance. Kidney Int. 14, 263-269.

Andreoli, T.E., Schafer, J.A., Troutman, S.L. and Watkins, M.L. (1979). Solvent drag component of Cl flux in superficial proximal straight tubules: evidence for a paracellular component of isotonic fluid absorption. Am. J. Physiol. 237, F455-F462.

Andreucci, V.E. (1978a). Surgery in the rat. In: Manual of Renal Micropuncture, edited by Andreucci, V.E. Naples, Idelson, pp. 50-86.

Andreucci, V.E. (1978b). Methods for inulin measurement in micro-samples. In: Manual of Renal Micropuncture, edited by Andreucci, V.E. Naples, Idelson, pp. 314-331.

Atlas, S.A., Sealey, J.E., Dharmgrongartama, B., Hesson, T.E. and Laragh, J.H. (1981). Detection and isolation of inactive, large molecular weight renin in human kidney and plasma. Hypertension 3 (Suppl. I), I-30 - I-40.

Aukland, K. (1968). Effect of adrenaline, noradrenaline, angiotensin and renal nerve stimulation on intrarenal distribution of blood flow in dogs. Acta. Physiol. Scand. 72, 498-509.

Ausiello, D.A., Kreisberg, J.I., Roy, C. and Karnovsky, M.J. (1980). Contraction of cultured rat glomerular cells of apparent mesangial origin after stimulation with angiotensin II and arginine vasopressin. J. Clin. Invest. 65, 754-760.

Baer, B.G. (1981). The contribution of prostaglandins to renal blood flow maintenance is determined by the level of activity of the renin-angiotensin system. Life Sci. 28, 587-593.

Bailie, M.D., Rector, F.C.,Jr. and Seldin, D.W. (1971). Angiotensin II in arterial and renal venous plasma and renal lymph in the dog. J. Clin. Invest. 50, 119-126.

Bakhle, Y.S. (1968). Conversion of angiotensin I to angiotensin II by cell-free extracts of dog lung. Nature 220, 919-920.

Bakhle, Y.S., Reynard, A.M. and Vane, J.R. (1969). Metabolism of the angiotensins in isolated perfused tissues. Nature 222, 956-959.

Baldamus, C.A., Heirholzer, K., Rumrich, G., Stolte, H., Uhlich, E., Ullrich, K.J. and Wiederholt, M. (1969). Natriumtransportt in den proximalen tubuli und den sammelrohren bei variation der natriumkonzentration im umgebenden interstitium. Pflugers Arch. 310, 354-368.

Bank, N. (1970). The regulation of renal sodium transport by intrarenal physical forces: A resume. Mount Sinai J. Med. 37 (4), 388-395.

Bank, N., Aynedjian, H.S., Bansal, V.K. and Goldman, D.M. (1970). Effect of acute hypertension on sodium transport by the distal nephron. Am. J. Physiol. 219, 275-280.

Bank, N., Aynedjian, H.S., and Wada, T. (1972). Effect of peritubular capillary perfusion rate on proximal sodium reabsorption. Kidney Int. 1, 397-405.

Bank, N., Koch, K.M., Aynedjian, H.S. and Aras, M. (1969). Effects of changes in renal perfusion pressure on the suppression of proximal tubular sodium reabsorption due to saline loading. J. Clin. Invest. 48, 271-283.

Barajas, L. (1970). The ultrastructure of the juxtaglomerular apparatus as disclosed by three-dimensional reconstructions from serial sections. J. Ultrastruct. Res. 33, 116-147.

Barbour, B.H., Gill, J.R.,Jr. and Barter, F.C. (1964). Effects of angiotensin on the toad skin. Proc. Soc. Exp. Biol. Med. 116, 806-808.

Barger, A.C., Berlin, R.D. and Tulenko, J.F. (1958). Infusion of aldosterone, 9-α-fluorohydrocortisone and antidiuretic hormone into the renal artery of normal and adrenalectomized, unanesthetized dogs: effect on electrolyte and water excretion. Endocrinology 62, 804-815.

Barger, A.C., Yates, F.E. and Rudolph, A.M. (1961). Renal haemodynamics and sodium excretion in dogs with graded valvular damage and in congestive heart failure. Am. J. Physiol. 200, 601-608.

Barraclough, M.A. (1965). Dose-dependent opposite effects of angiotensin on renal sodium excretion. Lancet II, 987-988.

Barraclough, M.A., Jones, N.F. and Marsden, C.D. (1967). Effect of angiotensin on renal function in the rat. Am. J. Physiol. 212, 1153-1157.

Barratt, L.J., Rector, F.C.,Jr., Kokko, J.P. and Seldin, D.W. (1974). Factors governing the transepithelial potential difference across the proximal tubule of the rat kidney. J. Clin. Invest. 53, 454-464.

Baum, T., Sybertz, E.J., Watkins, R.W., Ahn, H.S., Nelson, S., Eynon, E., Vander Vliet, G., Pula, K.K., Sabin, C., Desiderio, D.M., Becker, F.T. and Vemulapalli, S. (1983). Antihypertensive activity of SCH 31846, a non-sulphydryl angiotensin-converting enzyme inhibitor. J. Cardiovasc. Pharmacol. 5, 655-667.

Baylis, C. and Brenner, B.M. (1978). Modulation by prostaglandin synthesis inhibitors of the action of exogenous angiotensin II on glomerular ultrafiltration in the rat. Circ. Res. 43, 889-898.

Baylis, C., Deen, W.M., Myers, B.D. and Brenner, B.M. (1976). Effects of some vasodilator drugs on transcapillary fluid exchange in renal cortex. Am. J. Physiol. 230, 1148-1158.

Bell, P.D. and Navar, L.G. (1982). Intrarenal feedback control of glomerular filtration rate. Seminars in Nephrology 2, 289-301.

Bello-Reuss, E. (1980). Effects of catecholamines on fluid reabsorption by the isolated proximal convoluted tubule. Am. J. Physiol. 238, F347-F352.

Berry, C.A. (1983). Lack of effect of peritubular protein on passive NaCl transport in the rabbit proximal convoluted tubule. J. Clin. Invest. 71, 268-281.

Berry, C.A. and Cogan, M.G. (1981). Influence of peritubular protein on solute absorption in the rabbit proximal tubule: a specific effect on NaCl transport. J. Clin. Invest. 68, 506-516.

Berry, C.A. and Rector, F.C.,Jr. (1980). Active and passive sodium transport in the proximal tubule. Miner. Electrolyte Metab. 4, 149-160.

Berry, C.A., Warnock, D.G. and Rector, F.C.,Jr. (1978). Ion selectivity and proximal salt absorption. Am. J. Physiol. 235, F234-F245.

Bickerton, R.K. and Buckley, J. P. (1961). Evidence for a central mechanism in angiotensin induced hypertension. Proc. Soc. Exp. Biol. Med. 106, 834-836.

Bing, J. (1972). Relation between renin substrate and acute phase protein synthesis. Acta. Pathol. Microbiol. Scand. 80, 646-650.

Biron, P. and Campeau, L. (1971). Pulmonary and extrapulmonary fate of angiotensin I. Rev. Can. Biol. 30, 27-34.

Biron, P., Kiow, E., Nowaczynski, W., Brouillet, J. and Genest, J. (1961). The effects of intravenous infusion of valine-5 angiotensin II and other pressor agents on urinary electrolytes and corticosteroids, including aldosterone. J. Clin. Invest. 40, 338-347.

Biron, P., Meyer, P. and Panisset, J.C. (1968). Removal of angiotensin from the systemic circulation. Can. J. Physiol. Pharmacol. 46, 175-178.

Bishop, J.H.V., Green, R. and Thomas, S. (1978). Effects of glucose on water and sodium reabsorption in the proximal convoluted tubule of rat kidney. J. Physiol. 275, 481-493.

Blair-West, J.R. (1976). Renin-angiotensin system and sodium balance. Int. Rev. Physiol. 11, 95-143.

Blair-West, J.R., Coghlan, J.P., Denton, D.A., Funder, J.W., Scoggins, B.A. and Wright, R.D. (1971a). The effect of the heptapeptide (2-8) and hexapeptide (3-8) fragments of angiotensin on aldosterone secretion. J. Clin. Endocrinol. Metab. 32, 575-578.

Blair-West, J.R., Coghlan, J.P., Denton, D.A., Funder, J.W., Scoggins, B.A. and Wright, R.D. (1971b). Inhibition of renin secretion by systemic and intrarenal angiotensin infusion. Am. J. Physiol. 220, 1309-1315.

Blantz, R.C., Katz, M.A., Rector, F.C.,Jr. and Seldin, D.W. (1971). Measurement of intrarenal blood flow. II. Effect of saline diuresis in the dog. Am. J. Physiol. 220, 1914-1920.

Blantz, R.C., Konnen, K.S. and Tucker, B.J. (1976). Angiotensin II effects upon the glomerular microcirculation and ultrafiltration coefficient of the rat. J. Clin. Invest. 57, 419-434.

Bock, K.D., Dengler, H., Krecke, H.J. and Reichel, G. (1958). Untersuchungen uber die Wirkung von Synthetischen Hypertensin II auf Elektrolythaushalt, Nienfunktion und Kreislauf beim Menschen. Klin. Wochenschr. 36, 809-814.

Bolton, J.E., Munday, K.A., Parsons, B.J. and York, B.G. (1975). Effects of angiotensin II on fluid transport, transmural potential difference and blood flow by rat jejunum in vivo. J. Physiol. 253, 411-428.

Bonjour, J.P. and Malvin, R.L. (1969). Renal extraction of PAH, GFR and $U_{Na}V$ in the rat during infusion of angiotensin. Am. J. Physiol. 216, 554-562.

Bonnardeaux, J.L. and Regoli, D. (1974). Action of angiotensin on the heart. Can. J. Physiol. Pharmacol. 52, 50-60.

Bonvalet, J.P., Champion, M., Wanstok, F. and Berjal, G. (1977). Nephron filtration rate in conscious rats recovering from anaesthesia. Am. J. Physiol. 232, F329-F334.

Bouhnik, J., Clauser, E., Strosberg, D., Frenoy, J. P., Menard, J. and Corvol, P. (1981). Rat angiotensinogen and des (angiotensin I) angiotensinogen: purification, characterisation and partial sequencing. Biochemistry 20, 7010-7015.

Boulpaep, E.L. (1972). Permeability changes of the proximal tubule of NECTURUS during saline loading. Am. J. Physiol. 222, 517-529.

Braun-Menendez, E., Fasciolo, J.C., Leloir, L.F. and Munoz, J. (1940). The substance causing renal hypertension. J. Physiol. 98, 283-298.

Brenner, B.M., Badr, K.F., Schor, N. and Ichikawa, I. (1980). Hormonal influences on glomerular filtration. Miner. Electrolyte Metab. 4, 49-56.

Brenner, B.M., Falchuk, K.H., Keimowitz, R.I. and Berlinner, R.W. (1969). The relationship between peritubular capillary protein concentration and fluid reabsorption by the renal proximal tubule. J. Clin. Invest. 48, 1519-1531.

Brenner, B.M. and Troy, J.L. (1971). Postglomerular vascular protein concentration: Evidence for a causal role in governing fluid reabsorption and glomerulotubular balance by the renal proximal tubule. J. Clin. Invest. 50, 336-349.

Briggs, J.P. and Wright, F.S. (1979). Feedback control of glomerular filtration rate: site of the effector mechanism. Am. J. Physiol. 236, F40-F47.

Britton, S.L. (1981). Intrarenal vascular effects of angiotensin I and angiotensin II. Am. J. Physiol. 240, H914-H919.

Brooks, D.P., Chen, C.F., Chapman, B.J. and Munday, K.A. (1983). The effect of alterations in sodium chloride intake on the half-life and cardiovascular actions of angiotensin II in the rat. Mol. Physiol. 4, 67-73.

Brooks, V.L. and Malvin, R.L. (1980). Intracerebroventricular infusion of angiotensin II inhibits aldosterone secretion. Am. J. Physiol. 239, E447-E453.

Brown, A.J., Casals-Stenzel, J., Gofford, S., Lever, A.F. and Morton, J.J. (1981). Comparison of fast and slow pressor effects of angiotensin II in the conscious rat. Am. J. Physiol. 241, H381-H388.

Brown, G. P. and Douglas, J.G. (1982). Angiotensin II binding sites on isolated rat renal brush border membranes. Endocrinology 111, 1830-1836.

Brown, G.P. and Douglas, J.G. (1983). Angiotensin II-binding sites in rat and primate isolated renal tubular basolateral membranes. Endocrinology 112, 2007-2014.

Brown, J.J., Davies, D.L., Lever, A.F., Parker, R.A. and Robertson, J.I.S. (1966). The assay of renin in single glomeruli and the appearance of the juxtaglomerular apparatus in the rabbit following renal artery constriction. Clin. Sci. 30, 223-235.

Brown, J.J., Davies, D.L., Lever, A.F. and Robertson, J.I.S. (1963). Influence of sodium loading and sodium depletion on plasma-renin in man. Lancet II, 278-279.

Brown, J.J., Davies, D.L., Lever, A.F. and Robertson, J.I.S. (1964a). Variations in plasma renin concentration in several physiological and pathological states. Can. Med. Assoc. J. 90, 201-206.

Brown, J.J., Davies, D.L., Lever, A.F. and Robertson, J.I.S. (1964b). Influences of sodium deprivation and loading on the plasma-renin in man. J. Physiol. 173, 408-419.

Brunner, H.R., Gavras, H., Waeber, B., Kershaw, G.R., Turini, G.A., Vukovich, R.A., McKinstry, D.N. and Gavras, I. (1979). Oral angiotensin converting enzyme inhibitor in long term treatment of hypertensive patients. Ann. Int. Med. 90, 19-23.

Bruns, F.J., Alexander, E.A., Riley, A.L. and Levinsky, N.G. (1974). Superficial and juxtamedullary nephron function during saline loading in the dog. J. Clin. Invest. 53, 971-979.

Burg, M.B. and Orloff, J. (1968). Control of fluid absorption in the renal proximal tubule. J. Clin. Invest. 47, 2016-2024.

Burnett, J.C.,Jr., Haas, J.A. and Knox, F.G. (1982). Segmental analysis of sodium reabsorption during renal vein constriction. Am. J. Physiol. 243, F19-F22.

Burton, J., Cody, R.J.,Jr., Evin, G., Poulsen, K., Herd, J.A. and Haber, E. (1980). A substrate analog inhibitor of renin that is effective in vivo. Biochem. Biophys. Res. Comm. 97, 230-235.

Caldicott, W.J.H., Taub, K.J., Margulies, S.S. and Hollenberg, N.K. (1981). Angiotensin receptors in glomeruli differ from those in renal arterioles. Kidney Int. 19, 687-693.

Caldwell, P.R.B., Seegal, B.C., Hsu, K.C., Das, M. and Soffer, R.L. (1976). Angiotensin-converting enzyme: Vascular endothelial localisation. Science 191, 1050-1051.

Campbell, W.B., Brooks, S.N. and Pettinger, W.A. (1974). Angiotensin II- and angiotensin III- induced aldosterone release in vivo in the rat. Science 184, 994-996.

Campbell, W.B., Graham, R.M. and Jackson, E.K. (1979). Role of renal prostaglandins in sympathetically mediated renin release in the rat. J. Clin. Invest. 64, 448-456.

Caravaggi, A.M., Bianchi, G., Brown, J.J., Lever, A.F., Morton, J.J., Powell-Jackson, J.D., Robertson, J.I.S. and Semple, P.F. (1976). Blood pressure and plasma angiotensin II concentration after renal artery constriction and angiotensin infusion in the dog. Circ. Res. 38, 315-321.

Carmines, P.K., Rosivall, L., Till, M.F. and Navar, L.G. (1983). Renal hemodynamic effects of captopril in anesthetized sodium-restricted dogs: Relative contributions of prostaglandin stimulation and suppressed angiotensin activity. Renal Physiol. 6, 281-287.

Carretero, O., Berjak, B. and Houle, J.A. (1971). Renin isozymes of extrarenal origin. Am. J. Physiol. 220, 1468-1472.

Carretero, O. and Gross, F. (1967). Renin substrate in plasma under various experimental conditions in the rat. Am. J. Physiol. 213, 695-700.

Carriere, S., Boulet, P., Mathieu, A. and Brunette, M.G. (1972). Isotonic saline loading and intrarenal distribution of glomerular filtration in dogs. Kidney Int. 2, 191-196.

Carriere, S. and Friborg, J. (1969). Intrarenal blood flow and PAH extraction during angiotensin infusion. Am. J. Physiol. 217, 1708-1715.

Celio, M.R. and Inagami, T. (1981). Angiotensin II immunoreactivity co-exists with renin in the juxtaglomerular granular cells of the kidney. Proc. Natl. Acad. Sci. USA. 78, 3897-3900.

Chesley, L.C. (1974). Renin, angiotensin and aldosterone. Obstet. Gynecol. Ann. 3, 235-265.

Cheung, H.S. and Cushman, D.W. (1971). A soluble aspartate aminopeptidase from dog kidney. Biochim. Biophys. Acta. 293, 451-463.

Cheung, H.S. and Cushman, D.W. (1973). Inhibition of homogenous angiotensin-converting enzyme of rabbit lung by synthetic venom peptides of BOTHROPS jararaca. Biochim. Biophys. Acta. 293, 451-463.

Chiaraviglio, E. (1976). Effect of renin-angiotensin system on sodium intake. J. Physiol. 255, 57-66.

Chiu, A.T., Ryan, J.W., Stewart, J.M. and Dorer, F.E. (1976). Formation of angiotensin III by angiotensin-converting enzyme. Biochem. J. 155, 189-192.

Churchill, P.C. (1980). Effect of D-600 on inhibition of in vitro renin release in the rat by high extracellular potassium and angiotensin II. J. Physiol. 304, 449-458.

Churchill, P.C., Churchill, M.C., and McDonald, F.D. (1978). Renin secretion and distal tubule Na^+ in rats. Am. J. Physiol. 235, F611-F616.

Clappison, B.H., Anderson, W.P. and Johnston, C.I. (1981). Renal hemodynamics and renal kinins after angiotensin converting enzyme inhibition. Kidney Int. 20, 615-620.

Click, R.L., Joyner, W.L. and Gilmore, J.P. (1979). Reactivity of glomerular afferent and efferent arterioles in renal hypertension. Kidney Int. 15, 109-115.

Coelho, J.B. (1974). Sodium metabolism and intrarenal distribution of nephron glomerular filtration rates in the unanaesthetised rat. Proc. Soc. Exp. Biol. Med. 146, 225-231.

Cohen, S., Taylor, J.M., Murakami, K., Michelakis, A.M. and Inagami, T. (1972). Isolation and characterisation of renin-like enzymes from mouse submaxillary glands. Biochemistry 11; 4286-4293.

Conger, J.D., Bartoli, E. and Earley, L.E. (1976). A study in vivo of peritubular oncotic pressure and proximal tubular reabsorption in the rat. Clin. Sci. Mol. Med. 51, 379-392.

Cook, W.F. (1967). The detection of renin in juxtaglomerular cells. J. Physiol. 194, 73P-74P.

Cortney, M.A., Mylle, M., Lassiter, W.E. and Gottschalk, C.W. (1965). Renal tubular transport of water, solute and PAH in rats loaded with isotonic saline. Am. J. Physiol. 209, 1199-1205.

Corvol, P., Devaux, C., Ito, T., Sicard, P., Duccoux, J. and Menard, J. (1977). Large scale purification of hog renin: physicochemical characterisation. Circ. Res. 41, 616-622.

Coviello, A. and Crabbe, J. (1965). Effect of angiotensin II on active transport of sodium by toad bladder and skin. Biochem. Pharmacol. 14, 1739-1744.

Crocker, A. and Munday, K.A. (1967). Aldosterone and angiotensin action on water absorption in the rat jejunum. J. Physiol. 192, 36P.

Curran, P.E. and MacIntosh, J.R. (1962). A model system for biological water transport. Nature 193, 347-348.

Cushman, D.W. and Cheung, H.S. (1971a). Concentrations of angiotensin-converting enzyme in tissues of the rat. Biochim. Biophys. Acta. 250, 261-265.

Cushman, D.W. and Cheung, H.S. (1971b). Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem. Pharmacol. 20, 1637-1648.

Cushman, D.W. and Cheung, H.S. (1972). Studies in vitro of angiotensin-converting enzyme of lung and other tissues. In: Hypertension 1972, edited by Genest, J. and Koiw, E. Berlin, Springer-Verlag, pp 532-541.

Cushman, D.W., Ondetti, M.A. and Cheung, H.S. (1978). Substrate and inhibitor binding to angiotensin-converting enzyme (ACE): Role of the C-terminal dipeptide sequence (Abstr.) Fed. Proc. 37, 657.

Das, M., Hartley, J.L. and Soffer, R.L. (1977). Serum angiotensin-converting enzyme-Isolation and relationship to pulmonary enzyme. J. Biol. Chem. 252, 1316-1319.

Das, M. and Soffer, R.L. (1975). Pulmonary angiotensin-converting enzyme. J. Biol. Chem. 250, 6762-6768.

Davalos, M., Frega, N.S., Baker, B. and Leaf, A. (1978). Effect of exogenous and endogenous angiotensin II in the isolated perfused rat kidney. Am. J. Physiol. 235, F605-F610.

Davies, N.T., Munday, K.A. and Parsons, B.J. (1970). The effect of angiotensin on rat intestinal fluid transfer. J. Endocr. 48, 39-46.

Davis, J.O. (1973). The control of renin release. Am. J. Med. 55, 333-350.

Davis, J.O. and Freeman, R.H. (1976). Mechanisms regulating renin release. Physiol. Rev. 56, 1-56.

Day, R.P. and Luetscher, J.A. (1974). Big renin: a possible prohormone in kidney and plasma of a patient with Wilms' tumor. J. Clin. Endocrinol. Metab. 38, 923-926.

Day, R.P. and Reid, I.A. (1976). Renin activity in dog brain: enzymological similarity to cathepsin D. Endocrinology 99, 93-100.

De Bono, E., Lee, G.D.J., Mottram, F.R., Pickering, G.W., Brown, J.J., Keen, H., Peart, W.S. and Sanderson, P.H. (1963). The action of angiotensin in man. Clin. Sci. 25, 123-127.

De Champlain, J., Genest, J., Veyrat, R. and Boucher, R. (1966). Factors controlling renin in man. Arch. Intern. Med. 117, 355-363.

De Forrest, J.M., Waldron, T.L. and Antonaccio, M.J. (1982). Renal responses to captopril in conscious dogs pretreated with indomethacin. Am. J. Physiol. 243, F543-F548.

Dengler, H. (1956). Uber einen reinactigen wirkstoff in arterienextrakten. Arch. Exp. Path. Pharmakol. 227, 481-487.

de Rouffignac, C. and Bonvalet, J.P. (1970). Variations in GFR of single superficial and deep nephrons under various conditions of sodium intake in the rat. Pflugers Arch. 317, 141-156.

De Wardener, H.E. (1978). The control of sodium excretion. Am. J. Physiol. 235, F163-F173.

De Wardener, H.E., Mills, I.H., Clapham, W.F. and Hayter, C.J. (1961). Studies on the efferent mechanism of the sodium diuresis which follows the administration of intravenous saline in the dog. Clin. Sci. 21, 249-258.

Diamond, J.M. and Bossert, W.H. (1967). Standing-gradient osmotic flow. A mechanism for coupling of water and solute transport in epithelia. J. Gen. Physiol. 50, 2061-2083.

Di Bona, G.F. (1982). The functions of the renal nerves. Rev. Physiol. Biochem. Pharmacol. 94, 75-181.

Dirks, J.H., Cirksena, W.F. and Berliner, A.W. (1965). The effect of saline infusion on sodium reabsorption by the proximal tubule of the dog. J. Clin. Invest. 44, 1160-1170.

Di Salvo, Y., Peterson, A., Montefusco, C. and Menta, M. (1971). Intrarenal conversion of angiotensin I to angiotensin II in the dog. Circ. Res. 29, 398-406.

Dorer, F.E., Kahn, J.R., Lentz, K.E., Levine, M. and Skeggs, L.T. (1972). Purification and properties of angiotensin-converting enzyme from hog lung. Circ. Res. 31, 356-366.

Dorer, F.E., Kahn, J.R., Lentz, K.E., Levine, M. and Skeggs, L.T. (1974). Hydrolysis of bradykinin by angiotensin converting enzyme. Circ. Res. 34, 824-827.

Dorer, F.E., Kahn, J.R., Lentz, K.E., Levine, M. and Skeggs, L.T. (1976). Kinetic properties of pulmonary angiotensin converting enzyme-Hydrolysis of hippurylglycylglycine. Biochim. Biophys. Acta. 429, 220-228.

Douglas, J.G. and Brown, G.P. (1982). Effect of prolonged low dose infusion of angiotensin II and aldosterone on rat smooth muscle and adrenal angiotensin II receptors. Endocrinology 111, 988-992.

Douglas, J.G. and Catt, K.J. (1976). Regulation of angiotensin II receptors in the rat adrenal cortex by dietary electrolytes. J. Clin. Invest. 58, 834-843.

Dousa, T.P., Shaw, S.V., and Abboud, H.E. (1980). Potential role of cyclic nucleotides in glomerular pathophysiology. In: Advances in cyclic nucleotide Research, vol. 12, edited by Hamet, P. and Sands, H. New York, Raven, pp. 285-299.

Doyle, A.E., Louis, W.J., Jerums G. and Osborn, E.C. (1968). Metabolism and blood levels following infusion of a radioactive analog of angiotensin. Am. J. Physiol. 215, 164-168.

Dworkin, L.D., Ichikawa, I. and Brenner, B.M. (1983). Hormonal modulation of glomerular function. Am. J. Physiol. 244, F95-F104.

Dzau, V.J., Slater, E.E. and Haber, E. (1979). Complete purification of dog renal renin. Biochemistry 18, 5224-5228.

Earley, L.E. and Friedler, R.M. (1964). Observations on the mechanism of decreased tubular reabsorption of sodium and water during saline loading. J. Clin. Invest. 43, 1928-1937.

Earley, L.E. and Friedler, R.M. (1965). Changes in renal blood flow and possibly the intrarenal distribution of blood flow during the natriuresis accompanying saline loading. J. Clin. Invest. 44, 929-941.

Earley, L.E. and Schrier, R.W. (1973). Intrarenal control of sodium excretion by hemodynamic and physical factors. In: Handbook of Physiology. Renal Physiology, edited by Orloff, J. and Berliner, R.W. Washington, D.C. American Physiological Society, pp. 721-762.

Edelman, I.S. (1979). Mechanism of action of aldosterone: Energetic and permeability factors. J. Endocrinol. 81, 49P-53P.

Edelman, R. and Hartroft, P.M. (1961). Localisation of renin in the juxtaglomerular cells of the rabbit and dog through the use of the fluorescent-antibody technique. Circ. Res. 9, 1069-1077.

Edwards, R.M. (1983). Segmental effects of norepinephrine and angiotensin II on isolated renal microvessels. Am. J. Physiol. 244, F526-F534.

Eggena, P. Hidaka, H., Barrett, J.D. and Sambhi, M. P. (1978). Multiple forms of human plasma renin substrate. J. Clin. Invest. 1, 367-372.

Eliseeva, Y.E., Orekhovich, V.N., Pavlikhina, L.V. and Alexeenko, L.P. (1971). Carboxycathepsin - A key regulatory component of two physiological systems involved in regulation of blood pressure. Clin. Chim. Acta. 31, 413-419.

Erdos, E.G. (1975). Angiotensin I converting enzyme. Circ. Res. 36, 247-255.

Faarup, P. (1968). Renin location in different parts of the juxtaglomerular apparatus in the cat kidney. 2. Fraction of the afferent arteriole, the cell group of Goormaghtigh, the efferent arteriole and the glomerulus. Acta. Pathol. Microbiol. Scand. 72, 109-117.

Faarup, P. (1971). Morphological aspects of the renin-angiotensin system. Acta. Pathol. Microbiol. Scand. 222 (suppl), 1-96.

Fagard, R.H., Amery, A.K. and Lijnen, P.J. (1978). Renal responses to angiotensin II and 1-sar-8-ala-AII in sodium replete and deplete dogs. Pflugers Arch. 374, 199-204.

Farr, W.C. and Grupp, G. (1971). Ganglionic stimulation: mechanisms of the positive inotropic and chronotropic effects of angiotensin. J. Pharmacol. Exptl. Therap. 177, 48-55.

Farruggia, P., Sachs, S and Palaic, D. (1979). Effects of angiotensinase inhibitors on angiotensin receptors in rabbit aorta. Mol. Pharmacol. 15, 525-530.

Ferreira, S.H. (1965). A bradykinin-potentiating factor (BPF) present in the venom of BOTHROPS jararaca. Brit. J. Pharmacol. 24, 163-169.

Ferreira, S.H. (1966). Bradykinin-potentiating factor. In: Hypotensive Peptides, edited by Erdos, E.G., Back, N., Sicuteri, F. and Wilde, A.F. New York, Springer-Verlag, pp. 356-367.

Ferreira, S.H., Bartelt, D.C. and Greene, L.J. (1970). Isolation of bradykinin-potentiating peptides from BOTHROPS jararaca venom. Biochemistry 9, 2583-2593.

Ferreira, S.H. and Rocha e Silva, M. (1965). Potentiation of bradykinin and eledoisin by BPF (Bradykinin Potentiating Factor) from BOTHROPS jararaca venom. Experientia 21, 347-349.

Ferris, T.F., Gorden, P. and Mulrow, P.J. (1967). Rabbit uterus as a source of renin. Am. J. Physiol. 212, 698-670.

Fischer-Ferraro, C., Nahmod, V.E., Goldstein, D.J. and Finkielman, S. (1971). Angiotensin and renin in rat and dog brain. J. Exp. Med. 133, 353-361.

Fishman, M.G., Zimmerman, E.A. and Slater, E.E. (1981). Renin and angiotensin II: the complete system within the neuroblastoma and glioma. Science 214, 922-923.

Fitz, A. and Overturf, M. (1972). Molecular weight of human angiotensin I converting enzyme. J. Biol. Chem. 247, 581-584.

Fitzgibbons, J.P., Gennari, J., Garfinkel, H.B. and Cortell, S. (1974). Dependence of saline-induced natriuresis upon exposure of the kidney to the physical effects of extracellular fluid volume expansion. J. Clin. Invest. 54, 1428-1436.

Fitzsimons, J.T., Kucharczyk, J. and Richards, G. (1978). Systemic angiotensin-induced drinking in the dog: a physiological phenomenon. J. Physiol. 276, 435-448.

Foidart, J.B., Sraer, J., Dellarue, F., Mahieu, P.R. and Ardaillou, R. (1980). Evidence for mesangial glomerular receptors for angiotensin II linked to mesangial cell contractility. FEBS Lett. 121, 333-339.

Franklin, W.G., Peach, M.J. and Gilmore, J.P. (1970). Evidence for the renal conversion of angiotension I in the dog. Circ. Res. 27, 321-324.

Fray, J.C.S., Johnson, M.D. and Barger, A.C. (1977). Renin release and pressor response to renal arterial hypotension: effect of dietary sodium. Am. J. Physiol. 233, H191-H195.

Fray, J.C.S., Siwek, L.G., Strull, W.M., Steller, R.N. and Wilson, J.M. (1976). Influence of dietary sodium on renin activity and arterial pressure during anaesthesia. Am. J. Physiol. 231, 1185-1190.

Freedlender, A.E. and Goodfriend, T.C. (1977). Angiotensin recptors and sodium transport in renal tubules. Fed. Proc. 36, 481.

Freeman, R.H., Davis, J.O. and Lohmeier, T.E. (1975). Des-1-Asp-angiotensin II: Possible intrarenal role in homeostasis in the dog. Circ. Res. 37, 30-34.

Freeman, R.H., Davis, J.O. and Villarreal, D. (1984). Role of renal prostanglandins in the control of renin release. Circ. Res. 54, 1-9.

Freeman, R.H. and Rostorfer, H.H. (1972). Hepatic changes in renin substrate biosynthesis and alkaline phosphate activity in the rat. Am. J. Physiol. 223, 364-370.

Fromter, E., Rumrich, G. and Ullrich, K.J. (1973). Phenomenologic description of Na, Cl, and bicarbonate absorption from proximal tubules of rat kidney. Pflugers Arch. 343, 189-220.

Gagnon, J.A., Rice, M.K. and Flamenbaum, W. (1974). Effect of angiotensin converting enzyme inhibition on renal autoregulation. Proc. Soc. Exp. Biol. Med. 146, 414-418.

Galen, F.X., Devaux, C., Guyenne, T., Menard, J. and Corvol, P. (1979). Multiple forms of human renin: purification and characterisation. J. Biol. Chem. 254, 4848-4855.

Ganong, W.F. and Mulrow, P.J. (1958). Rate of change of sodium and potassium excretion after injection of aldosterone into the aorta and renal artery of the dog. Am. J. Physiol. 195, 337-342.

Ganten, D., Granger, P., Ganten, U., Boucher, R. and Genest, J. (1972). An intrinsic renin-angiotensin system in the brain. In: Hypertension 1972, edited by Genest, J. and Koiw, E. Berlin, Springer-Verlag, pp. 423-432.

Ganten, D., Hayduk, K., Brecht, H.M., Boucher, R. and Genest, J. (1970). Evidence of renin release or production in splanchnic territory. Nature 226, 551-552.

Ganten, D., Minnich, J.L., Granger, P., Hayduk, K., Brecht, H.M., Barbeau, A., Boucher, R. and Genest, J. (1971). Angiotensin-forming enzyme in brain tissue. Science 173, 64-65.

Ganten, D. and Speck, G. (1978). The brain renin-angiotensin system: a model for the synthesis of peptides in the brain. Biochem. Pharmol. 17, 2379-2389.

Gardes, J., Bouhnik, J., Clauser, E., Corvol, P. and Menard, J. (1982). Role of angiotensinogen in blood pressure homeostasis. Hypertension 4, 185-189.

Gavras, H., Brunner, H.R., Turini, G.A., Kershaw, G.R., Tifft, C.P., Cuttelod, S., Gavras, I., Vukovich, R.A. and McKinstry, D.N. (1978). Antihypertensive effect of the oral angiotensin converting enzyme inhibitor SQ 14,225 in man. N. Engl. J. Med. 298, 991-995.

Gavras, H., Brunner, H.R., Vaughan, E.D.,Jr. and Laragh, J. H. (1973). Angiotensin-sodium interaction in blood pressure maintenance of renal hypertensive and normotensive rats. Science 180, 1369-1372.

Gertz, K.H. (1963). Transtubulare Natriumchloridflusse und Permeabilitat fur Nichtelektrolyte im proximalen und distalen Konvolut der Ratteniere. Pflugers Arch. 276, 336-356.

Gertz, K.H. and Boylan, J.W. (1973). Glomerular tubular balance. In: Handbook of Physiology, Renal Physiology, edited by Orloff, J. and Berliner, R.W. Washington, D.C. American Physiological Society, pp. 763-790.

Giebisch, G., Klose, R.M. and Windhager, E.E. (1964). Micropuncture study of hypertonic sodium chloride loading in the rat. Am. J. Physiol. 206, 687-693.

Glenner, G.G., McMillan, P.H. J. and Folk, L.F. (1962). A mammalian peptidase specific for the hydrolysis of N-terminal L-glutamyl and aspartyl residues. Nature 194, 867.

Goldblatt, H., Lynch, J., Hanzel., R.F. and Summerville, W.W. (1934). The production of persistent elevation of systolic blood pressure by means of renal ischaemia. J. Exp. Med. 59, 347-379.

Goldstein, D.J., Diaz, A., Finkielman, G., Nahmod, V.E. and Fischer-Ferraro, C. (1972). Angiotensin activity in rat and dog brain. J. Neurochem. 19, 2451-2452.

Goodfriend, T.L. and Peach, M.J. (1975). Angiotensin III; (des-aspartic acid[1]) angiotensin II. Evidence and speculation for its role as an important agonist in the rein-angiotensin system. Circ. Res. 36-37 (suppl. I), I-38-I-48.

Goormaghtigh, N. (1939). Existance of an endocrine gland in the media of the renal arterioles. Proc. Soc. Exp. Biol. Med. 42, 688-689.

Gordon, D.B., Sachin, I.N. and Dodd, V.N. (1976). Heterogeneity of renin substrate in human plasma: effect of pregnancy and of contraceptives. Proc. Soc. Exp. Biol. Med. 153, 314-318.

Gottschalk, C.W. and Mylle, M. (1959). Micropuncture study of the mammalian urinary concentrating mechanism: evidence for the counter-current hypothesis. Am. J. Physiol. 196, 927-936.

Gould, A.B. and Green D. (1971). Kinetics of the human renin and renin substrate reaction. Cardiovasc. Res. 5, 86-89.

Gould, A.B. and Goodman, S.A. (1970). The effect of hypoxia on the renin-angiotensin system. Lab. Invest. 22, 443-457.

Gould, A.B., Skeggs, L.T. and Kahn, J.R. (1964). Presence of renin activity in blood vessel walls. J. Exp. Med. 119, 389-399.

Gould, A.B., Skeggs, L.T. and Kahn, J.R. (1966). Measurement of renin and substrate concentrations in human serum. Lab. Invest. 15, 1802-1813.

Graeff, F.G., Ferreira, S.H., Corrado, A.P. and Rocha e Silva, M. (1965). Potentation of the cerebral vascular action of bradykinin by the 'Bradykinin Potentiating Factor' (BPF) in the dog. Experientia 21, 607-608.

Grandchamp, A. and Boulpaep, E.L. (1974). Pressure control of sodium reabsorption and intercellular backflux across proximal kidney tubule. J. Clin. Invest. 54, 69-82.

Granger, P., Dalheim, H. and Thurau, K. (1969). Renin-angiotensinase and converting enzyme- Bestimmungen en einzelnen mikrodissezierten juxtaglomerularen apparaten und teilen des nephrons. Pflugers Arch. 312, R87.

Green, R. and Giebisch, G. (1984). Luminal hypotonicity: a driving force for fluid absorption from the proximal tubule. Am. J. Physiol. 246, F167-F174.

Greene, L.J., Stewart, J.M. and Ferreira, S.H. (1969). Bradykinin-potentiating peptides from the venom of BOTHROPS jararaca. Pharmacol. Res. Commun. 1, 159-160.

Gross, D.M., Sweet, C.S., Ulm, E.H., Backlund, E.P., Morris, A.A., Weitz, D., Bohn, D.L., Wenger, H.C., Vassil, T.C. and Stone, C.A. (1981). Effect of N-[(S)-1-carboxy-3-phenylpropyl] -L-Ala-L-Pro and its ethyl ester (MK 421) on angiotensin converting enzyme in vitro and angiotensin I pressor responses in vivo. J. Pharmacol. Exp. Ther. 216, 552-557.

Gross, F., Schaechtelin, G., Zeigler, M. and Berger, M. (1964). A renin-like substance in the placenta and uterus of the rabbit. Lancet. I, 914-916.

Guyton, A.C., Langston, J.B. and Navar, L.G. (1964). Theory for renal autoregulation by feedback at the juxtaglomerular apparatus. Circ. Res. 15 (suppl. I), 187-196.

Gyory, A.Z. (1971). Reexamination of the split oil droplet method as applied to kidney tubules. Pflugers Arch. 324, 328-343.

Haberle, D.A. and Baeyer, H. Von. (1983). Characteristics of glomerulotubular balance. Am. J. Physiol. 244, F355-F366.

Haberle, D.A. and Davis, J.M. (1982). Chronic salt loading: Effects on plasma volume and regulation of glomerular filtration rate in Wistar rats. Klin. Wochenschr. 60, 1245-1248.

Hall, J.E. (1982). Regulation of renal hemodynamics. Int. Rev. Physiol. 26, 243-321.

Hall, J.E., Coleman, T.G., Guyton, A.C., Balfe, J.W. and Salgado, H.C. (1979a). Intra-renal role of angiotensin II (des-Asp^1)-angiotensin II. Am. J. Physiol. 236, F252-F259.

Hall, J.E. and Granger, J.P. (1983). Renal hemodynamic action of angiotensin II: interaction with tubuloglomerular feedback. Am. J. Physiol. 245, F166-F173.

Hall, J.E., Guyton, A.C. and Cowley, A.W.,Jr. (1977a). Dissociation of renal blood flow and filtration rate autoregulation by renin depletion. Am. J. Physiol. 232, F215-F221.

Hall, J.E., Guyton, A.C., Jackson, T.E., Coleman, T.G., Lohmeier, T.E. and Trippodo, N.C. (1977c). Control of glomerular filtration rate by the renin-angiotensin system. Am. J. Physiol. 233, F366-F372.

Hall, J.E., Guyton, A.C., Smith, M.J.,Jr. and Coleman, T.G. (1979b). Chronic blockade of angiotensin II formation during sodium deprivation. Am. J. Physiol. 237, F424-F432.

Hall, J.E., Guyton, A.C., Smith, M.J.,Jr. and Coleman, T.G. (1980a). Blood pressure and renal function during chronic changes in sodium intake: role of angiotensin. Am. J. Physiol. 239, F271-F280.

Hall, J.E., Guyton, A.C., Smith, M.J.,Jr. and Coleman, T.G. (1980b). Long-term regulation of arterial pressure, glomerular filtration and renal sodium reabsorption by angiotensin II in dogs. Clin. Sci. 59, 87s-90s.

Hall, J.E., Guyton, A.C., Trippodo, N.C., Lohmeier, T.E., McCaa, R.E. and Cowley, A.W.,Jr. (1977b). Intrarenal control of electrolyte excretion by angiotensin II. Am. J. Physiol. 232, F538-F544.

Harris, P.J. and Munro, J.O. (1984). Reversal by angiotensins II and III of the effects of converting enzyme inhibition on renal electrolyte excretion in rats. J. Physiol. 351, 491-500.

Harris, P.J. and Navar, L.G. (1985). Tubular transport responses to angiotensin. Am. J. Physiol. 248, F621-F630.

Harris, P.J., Navar, L.G. and Ploth, D.W. (1984). Evidence for angiotensin-stimulated proximal tubular sodium reabsorption in normotensive and hypertensive rats: effect of acute administration of captopril. Clin. Sci. 66, 541-544.

Harris, P.J. and Young, J.A. (1977). Dose-dependent stimulation and inhibition of proximal tubular sodium reabsorption by angiotensin II in the rat kidney. Pflugers Arch. 367, 295-297.

Hartle, D.K. and Brody, M.J. (1984). The angiotensin II pressor system of the rat forebrain. Circ. Res. 54, 355-356.

Hartroft, P.M., Sutherland, L.E. and Hartroft, W.S. (1964). Juxtaglomerular cells as the source of renin: Further studies with the fluorescent antibody technique and the effect of passive transfer of antirenin. Can. Med. Assoc. J. 90, 163-166.

Hartupee, D.A., Burnett, J.C.,Jr., Mertz, J.I. and Knox, F.G. (1982). Acetylcholine-induced vasodilation without natriuresis during control of interstitial pressure. Am. J. Physiol. 243, F325-F329.

Hasegawa, H., Nasjletti, A., Rice, K., Masson, G.M.C. (1973). Role of pituitary and adrenals in the regulation of plasma angiotensinogen. Am. J. Physiol. 225, 1-6.

Hatt, P.Y. (1967). The juxtaglomerular apparatus. In: Ultrastructure of the Kidney, edited by Dalton, A.J. and Haguenau, F. New York, Academic Press, pp. 101-141.

Hauger, R.L., Aguilera, G. and Catt, K.J. (1978). Angiotensin II regulates its receptor sites in the adrenal glomerulosa zone. Nature 271, 176-178.

Healy, J.K., Barcena, C., O'Connell, J.M. and Schreiner, G.E. (1965). Renal and pressor action of angiotensin in the normal dog. Am. J. Physiol. 208, 1093-1099.

Healy, J.K., and Douglas, J.B. (1968). The effect of angiotensin on renal carbonic anhydrase. Biochim. Biophys. Acta. 151, 520-522.

Healy, J.K. and Elliott, A.J. (1970). The effect of a natriuretic dose of angiotensin on rabbit kidney composition. Clin. Sci. 38, 727-739.

Healy, J.K. and Elliott, A.J. (1971). Angiotensin-induced renal changes without pressor response. Clin. Res. 19, 532.

Healy, J.K., Fraser, P.A. and Young, J.A. (1976). Inhibition of sodium transport by angiotensin II in the main duct of the rabbit mandibular gland isolated and perfused in vitro. Pflugers Arch. 363, 69-73.

Heel, R.C., Brogden, R.N., Speight, T.M. and Avery, G.S. (1980). Captopril: a preliminary review of its pharmacological properties and therapeutic efficacy. Drugs 20, 409-452.

Helmer, O.M. (1955). A factor in plasma that enhances contraction produced by angiotensin on rabbit aortic strips (abstr.). Fed. Proc. 14, 225.

Helmer, O.M. and Griffith, R.S. (1952). The effect of administration of estrogens on the renin substrate (hypertensinogen) content of rat plasma. Endocrinology 51, 421-426.

Helmer, O.M. and Judson, W.E. (1967). Influence of high renin substrate levels on renin-angiotensin system in pregnancy. Am. J. Obstet. Gynecol. 99, 9-17.

Hess, R. (1965). Arylamidase activity related to angiotensinase. Biochim. Biophys. Acta. 99, 316-324.

Hierholzer, K., Kawamura, S., Seldin, D.W., Kokko, J.P. and Jacobson, H.R. (1980). Reflection coefficients of various substration across superficial and juxtamedullary proximal convoluted segments of rabbit nephrons. Miner. Electrolyte Metab. 3, 172-180.

Hilgenfeldt, U. and Hackenthal, E. (1979). Purification and characterisation of rat angiotensinogen. Biochim. Biophys. Acta. 579, 375-385.

Hilgenfeldt, U. and Hackenthal, E. (1982). Separation and characterisation of two different species of rat angiotensinogen. Biochim. Biophys. Act. 708, 335-342.

Hirose, S., Yokosawa, H. and Inagami, T. (1978). Immunochemical identification of renin in rat brain and distinction from acid proteases. Nature 274, 392-393.

Hodari, A.A., Carretero. O.A. and Hodgkinson, C.P. (1969). Uterine production of renin in normal and nephrectomised dogs. Obstet. Gynecol. 34, 358-362.

Hodge, R.L., Ng, K.K.F. and Vane, J.R. (1967). Disappearance of angiotensin from the circulation of the dog. Nature 215, 138-141.

Hofbauer, K.G., Konrads, A., Schwartz, K. and Werner, U. (1978). Role of cyclic AMP in the regulation of renin release from the isolated perfused rat kidney. Klin. Wochenschr., Suppl. 1, 56, 51-59.

Hofbauer, K.G., Zschiedrich, H. and Gross, F. (1976). Regulation of renin release and intrarenal formation of angiotensin. Studies in the isolated perfused rat kidney. Clin. Exp. Pharmacol. Physiol. 3, 73-93.

Hollenberg, N.K. (1979). Pharmacologic interruption of the renin-angiotensin system. Ann. Rev. Pharmacol. Toxicol. 19, 559-582.

Hollenberg, N.K. (1982). Converting enzyme inhibitors: implications for renal perfusion and function. Clin. Exp. Pharmacol. Physiol. Suppl. 7, 73-79.

Hollenberg, N.K., Chenitz, W.R., Adams, D.F. and Williams, G.H. (1974). Reciprocal influence of salt intake on adrenal glomerulosa and renal vascular responses to angiotensin II in normal man. J. Clin. Invest. 54, 34-42.

Hollenberg, N.K., Williams, G.H., Burger, B., Chenitz, W., Hoosmand, I. and Adams, D.F. (1976). Renal blood flow and its response to angiotensin II. An interaction between oral contraceptive agents, sodium intake and the renin-angiotensin system in healthy young women. Circ. Res. 38, 35-40.

Holmberg, L., Kokko, J.P. and Jacobsen, H.R. (1981). Determination of chloride and bicarbonate permeabilities in proximal convoluted tubules, Am. J. Physiol. 241, F386-F394.

Horky, K., Rojo-Ortega, J.M., Boucher, R. and Genest, J. (1973). Role of renal lymph in relation to the renin-angiotensin system in the rat. Physiol. Bohemoslov. 22, 1-9.

Horky, K. Rojo-Ortega, J.M., Rogriguez, J., Boucher, R. and Genest, J. (1971). Renin, renin substrate and angiotensin I-converting enzyme in the lymph of rats. Am. J. Physiol. 220, 307-311.

Hornych, H., Beaufils, M. and Richet, G. (1972). The effect of exogenous angiotensin on superficial and deep glomeruli in the rat kidney. Kidney Int. 2, 336-343.

Hornych, H. and Richet, G. (1977). Dissociated effect of sodium intake on glomerular and pressor responses to angiotensin. Kidney Int. 11, 28-34.

Huang, W.-C., Ploth, D.W., Bell, P.D., Work, J. and Navar, L.G. (1981). Bilateral renal function responses to converting enzyme inhibitor (SQ20,881) in two-kidney one-clip hypertensive rats. Hypertension 3, 285-293.

Huggins, C.G., Corcoran, R.J., Gordon, J.S., Henry, H.W. and John, J. P. (1970). Kinetics of the plasma and lung angiotensin I converting enzyme. Circ. Res. 26-27 (suppl. I), I-93-I-101.

Ichikawa, I and Brenner, B.M. (1979). Local intrarenal vasoconstrictor-vasodilator interactions in mild partial ureteral obstruction. Am. J. Physiol. 236, F131-F140.

Ichikawa, I and Brenner, B.M. (1980). Importance of efferent arteriolar vascular tone in regulation of proximal tubular fluid reabsorption and glomerular-tubular balance in the rat. J. Clin. Invest. 65, 1192-1201.

Ichikawa, I., Miele, J.F. and Brenner, B.M. (1979). Reversal of renal cortical actions of angiotensin II by verapamil and manganese. Kidney Int. 16, 137-147.

Igic, R., Erdos, E.G., Yeh, H.S.J., Sorrells, K. and Nakajima, T. (1972). Angiotensin I converting enzyme of the lung. Circ. Res. 31 (suppl II), II-51-56.

Igic, R., Nakajima, T., Yeh, H.S.J., Sorrells, K. and Erdos, E.G. (1973). In: Pharmacology and the Future of Man, vol 5, edited by Acheson, G. Basel, Karger, pp. 307-319.

Imai, M. and Kokko, J.P. (1972). Effect of peritubular protein concentration on reabsorption of sodium and water in isolated perfused proximal tubules. J. Clin. Invest. 51, 314-325.

Imai, M. and Kokko, J.P. (1974). Transtubular oncotic pressure gradients and net fluid transport in isolated proximal tubules. Kidney Int. 6, 138-145.

Inagami, T., Celio, M.R., Clemens, D., Lau, D., Takii, Y., Kasselberg, A.G. and Hirose, S. (1980). Renin in rat and mouse brain: immunohistochemical identification and localisation. Clin. Sci. 59 (suppl. 6), 49s-51s.

Inagami, T. and Murakami, K. (1977). Pure renin: isolation from hog kidney and characterisation. J. Biol. Chem. 252, 2978-2983.

Inagami, T. and Murakami, K. (1980). Prorenin. Biomed. Res. I, 456-475.

Inagami, T., Okamura, T., Hirose, S., Clemens, D., Celio, M.R., Naruse, K., Takii, Y. and Yokosawa, H. (1982). Identification , characterisation and evidence for interneuronal function of renin in the brain and neuroblastoma cells. In: The Renin Angiotensin System in the Brain, edited by Ganten, D., Printz, M., Phillips, M.I. and Scholkens, B.A. Heidelberg, Springer-Verlag, pp. 64-75.

Itskovitz, H.D. and McGiff, J.C. (1974). Hormonal regulation of the renal circulation. Circ. Res. 34 (suppl. I), 65-73.

Itskovitz, H.D. and Miller, L.D. (1966). Renal angiotensinase activity. Its localisation and the effects of mercury. Circ. Res. 19, 507-513.

Itskovitz, H.D. and Miller, L.D. (1967). Differential studies of angiotensinase activity from several tissue sources. Am. J. Med. Sci. 254, 659-666.

Jacobsen, H.R., Kokko, J.P., Seldin, D.W. and Holmberg, C. (1982). Lack of solvent drag of NaCl and $NaHCO_3$ in rabbit proximal tubules. Am. J. Physiol. 243, F342-F348.

Jamison, R.L. and Lacy, F.B. (1971). Effect of saline infusion on superficial and juxtamedullary nephrons in the rat. Am. J. Physiol. 221, 690-697.

Jensen, P.K. and Steven, K. (1977). Angiotensin II induced reduction of peritubular capillary diameter in the rat kidney. Pflugers Arch. 371, 245-250.

Johns, E.J. (1980). A comparison of the ability of two angiotensin II receptor blocking drugs, 1-Sar-8-Ala angiotensin II and 1-Sar-8-Ile angiotensin II, to modify the regulation of glomerular filtration rate in the cat. Br. J. Pharmacol. 71, 499-506.

Johnson, D.C. and Ryan, J.W. (1968). Degradation of angiotensin II by a carboxypeptidase of rabbit liver. Biochim. Biophys. Acta. 160, 196-203.

Johnson, M.D. and Malvin, R.L. (1977). Stimulation of renal sodium reabsorption by angiotensin II. Am. J. Physiol. 232, F298-F306.

Johnston, C.I., Mendelsohn, F.A.O. and Doyle, A.E. (1972). Metabolism of angiotensin II in sodium depletion and hypertension in humans. Circ. Res. 30 (suppl. II), II-202-II-213.

Johnston, C.I., Mendelsohn, F.A.O., Hutchinson, J.H. and Morris, B. (1973). Composition of the juxtaglomerular granules isolated from rat kidney cortex. In: Mechanisms of Hypertension, edited by Sambhi, M.P.S. Amsterdam, Excerpta Medica, pp. 238-248.

Jones, S.M., Torretti, J., Williams, J.S. and Weinberger, S.F. (1979). Regional renin release by the cat kidney in vitro and in vivo. Am. J. Physiol. 237, F186-F195.

Kakimoto, T., Oshima, G., Yeh, H.S.J. and Erdos, E.G. (1973). Purification of lysosomal prolylcarboxypeptidase angiotensinase C. Biochim. Biophys. Acta 302, 178-182.

Kaloyanides, G.J., Bastron, R.D. and DiBona, G.F. (1974). Impaired autoregulation of blood flow and glomerular filtration rate in the isolated dog kidney depleted of renin. Circ. Res. 35, 400-412.

Kaloyanides, G.J. and DiBona, G.F. (1976). Effects of an angiotensin II antagonist on autoregulation in the isolated dog kidney. Am. J. Physiol. 230, 1078-1083.

Kato, H. and Suzuki, T. (1969). Bradykinin-potentiating peptides from the venom of AGKISTRODON halys blomhoffi. Experientia 25, 694-695.

Kato, H. and Suzuki, T. (1971). Bradykinin-potentiating peptides from the venom of AGKISTRODON halys blomhoffi: Isolation of five bradykinin potentiators and the amino acid sequences of two of them, potentiators B and C. Biochemistry 10, 972-980.

Keeton, T.K. and Campbell, W.B. (1980). The pharmacological alteration of renin release. Pharmacol. Rev. 32, 81-227.

Keil, L.C., Summy-Long, J. and Severs, W.B. (1975). Release of vasopressin by angiotensin II. Endocrinology 96, 1063-1065,

Khairallah, P.A., Bumpus, F.M., Page, I.H. and Smeby, R.R. (1963). Angiotensinase with a high degree of specificity in plasma and red cells. Science 140, 672-674.

Khairallah, P.A. and Page, I.H. (1967). Plasma angiotensinases. Biochem. Med. 1, 1-8.

Kiil, F. (1982). Mechanism of glomerulotubular balance: the whole kidney appraoch. Renal Physiol. 5, 209-221.

Kimbrough, H.M.,Jr., Vaughan, E.D.,Jr., Carey, R.M. and Ayers, C.R. (1977). Effect of intrarenal angiotensin II blockade on renal function in conscious dogs. Circ. Res. 40, 174-178.

Kinney, M.J. and Di Scala, V.A. (1974). Volume expansion and intrarenal blood flow of normal and salt-deprived rats. Am. J. Physiol. 227, 652-656.

Knox, F.G. and Haas, J.A. (1982). Factors influencing renal sodium reabsorption in volume expansion. Rev. Physiol. Biochem. Pharmacol. 92, 75-113.

Knox, F.G., Mertz, J.I., Burnett, J.C.Jr., and Haramati, A. (1983). Role of hydrostatic and oncotic pressures in renal sodium reabsorption. Circ. Res. 52, 491-500.

Kokko, J.P. (1973). Proximal tubule potential difference dependence on glucose, HCO_3 and amino acids. J. Clin. Invest. 52, 1362-1367.

Kokko, J.P., Burg, M.B. and Orloff, J. (1971). Characteristics of NaCl and water transport in the renal proximal tubule. J. Clin. Invest. 50, 69-76.

Krahe, P., Hofbauer, K.G. and Gross, F. (1970). Effects of endogenous renin on the function of the isolated kidney. Life Sciences 9, 1317-1320.

Krahe, P., Hofbauer, K.G. and Gross, F. (1981). Effects of angiotensin infusion on the isolated rabbit kidney. Proc. Soc. Exp. Biol. Med. 137, 1324-1327.

Krakoff, L.R. and Eisenfeld, A.J. (1977). Hormonal control of plasma renin substrate. Circ. Res. 41 (suppl. II), 43-46.

Kyte, J. (1976). Immunoferritin determination of the distribution of (Na^+ and K^+)-ATPase over the plasma membranes of renal convoluted tubules. II. Proximal segment. J. Cell Biol. 68, 304-318.

Landwehr, D.M., Klose, R.M. and Giebisch, G. (1967). Renal tubular sodium and water reabsorption in the isotonic sodium chloride-loaded rat. Am. J. Physiol. 212, 1327-1333.

Lanzillo, J.J. and Fanburg, B.L. (1974). Membrane-bound angiotensin-converting enzyme from rat lung. J. Biol. Chem. 249, 2312-2318.

Lanzillo, J.J. and Fanburg, B.L. (1976). Angiotensin I-converting enzyme from guinea pig lung and serum. A comparison of some kinetic and inhibitory properties. Biochim. Biophys. Acta 445, 161-168.

Lanzillo, J.J. and Fanburg, B.L. (1977). Angiotensin I converting enzyme from human plasma. Biochemistry 16, 5491-5495.

LaPointe, J.Y., Laprade, R. and Cardinal, J. (1983). Transepithelial and ratio of luminal to basolateral electrical resistance of rabbit proximal convoluted tubule perfused in vitro. Kidney Int. 23, 261A.

Laragh, J.H., Angers, M., Kelly, W.G. and Lieberman, S. (1960). The effect of epinephrine, norepinephrine, angiotensin II, and others on the secretory rate of aldosterone in man. J. Am. Med. Assoc. 174, 234-240.

Laragh, J.J. and Sealey, J.E. (1973). The renin-angiotensin-aldersterone hormonal system and regulation of sodium, potassium and blood pressure homeostasis. In: Handbook of Physiology. Renal Physiology, edited by Orloff, J. and Berliner, R.W. Washington, D.C. American Physiology Society, pp. 831-908.

Latta, H. (1973). Ultrastructure of the glomerulus and juxtaglomerular apparatus. In: Handbook of Physiology. Renal Physiology, edited by Orloff, J. and Berliner, R.W. Washington, D.C. American Physiological Society, pp. 1-29.

Leary, W.P. and Ledingham, J.G. (1969). Removal of angiotensin by isolated perfused organs of the rat. Nature 222, 959-960.

Leary, W.P. and Ledingham, J.G. (1970a). Renal and hepatic inactivation of angiotensin in rats. Influence of sodium balance and renal artery compression. Clin. Sci. 38, 573-582.

Leary, W.P. and Ledingham, J.G. (1970b). Inactivation of angiotensin II analogues by isolated perfused rat liver and kidney. Nature 227, 178-179.

Leckie, B.J. (1981). Inactive renin: an attempt at a perspective. Clin. Sci. 60, 119-130.

Leckie, B.J. and McConnell, A. (1975). Activation of renin in human plasma and in rabbit kidney extracts. J. Endocrinol. 65, 7P.

Ledingham, J.G. and Leary, W.P. (1974). Catabolism of angiotensin II. In: Angiotensin (Handbuch der exp. pharmakol. vol. 37), Berlin, Springer-Verlag, pp. 111-125.

Lee, H.J., Larue, J.N. and Wilson, I.B. (1971). Angiotensin converting enzyme from porcine plasma. Biochim. Biophys. Acta 235, 521-528.

Leloir, L.F., Munoz, J.M., Braun-Menendez, E. and Fasciolo, J.C. (1940). La secrecion de renina y la formacion de hipertensina. Rev. Soc. Argent. Biol. 16, 75-80.

Leloir, L.F., Munoz, J.M., Taquini, A.C., Braun-Menendez, E. and Fasciolo, J.C. (1942). La formacion del hipertensinogeno. Rev. Argent. Cardiol. 9, 269-277.

Le Morvan, P., Palaic, D. and Ferguson D. (1977). Angiotensinase activity and angiotensin receptor binding in guinea pig aorta. Can. J. Physiol. Pharmacol. 55, 652-657.

Levens, N.R., Munday, K.A., Parsons, B.J., Poat, J.A. and Stewart, C.P. (1979). Noradrenaline as a possible mediator of the actions of angiotensin on fluid transport by rat jejunum in vivo. J. Physiol. 286, 351-360.

Levens, N.R., Peach, M.J. and Carey, R.M. (1981a). Role of the intrarenal renin-angiotensin system in the control of renal function. Circ. Res. 48, 157-167.

Levens, N.R., Peach, M.J., Carey, R.M., Poat, J.A. and Munday, K.A. (1981b). Changes in an electroneutral transport process mediated by angiotensin II in the rat distal colon in vivo. Endocrinology 108, 1497-1504.

Levens, N.R., Peach, M.J., Carey, R.M., Poat, J.A. and Munday, K.A. (1981c). Response of rat jejunum to angiotensin II: role of norepinephrine and prostaglandins. Am. J. Physiol. 240, G17-G24.

Levens, N.R., Peach, M.J., Vaughan, E.D.,Jr. and Carey, R.M. (1981d). Demonstration of a primary antidiuretic action of angiotensin II: Effects of intrarenal converting enzyme inhibition in the conscious dog. Endocrinology 108, 318-330.

Levine, M., Dorer, F.E., Kahn, J.R., Lentz, K.E. and Skeggs, L.T. (1970). Chemical determination of hog kidney renin by an automated method. Anal. Biochem. 34, 366-375.

Levinsky, N.G. and Lalone, R.C. (1963). The mechansim of sodium diuresis after saline infusion in the dog. J. Clin. Invest. 42, 1261-1267.

Lewy, J.E. and Windhager, E.E. (1968). Peritubular control of proximal tubular fluid reabsorption in the rat kidney. Am. J. Physiol. 214, 943-954.

Leyssac, P.P. (1964). The in vivo effect of angiotensin on the proximal tubular reabsorption of salt in rat kidneys. Acta Physiol. Scand. 62, 436-448.

Leyssac, P.P. (1965). The in vivo effect of angiotensin and noradrenaline on the proximal tubular reabsorption of salt in mammalian kidneys, Acta. Physiol. Scand. 64, 167-175.

Leyssac, P.P., Kristensen, L.O., Christensen, P. and Frederiksen, O. (1974). The effect of angiotensin on isosmotic fluid absorption by the rabbit gall-bladder in vitro. Acta. Physiol. Scand. 92, 508-516.

Liu, F-Y., Cogan, M.G. and Rector, F.C.,Jr. (1984). Axial heterogeneity in the rat proximal convoluted tubule. II. Osmolality and osmotic water permeability. Am. J. Physiol. 247, F822-F826.

Lohmeier, T.E. and Cowley, A.W. (1979). Hypertensive and renal effects of chronic low level intrarenal angiotensin infusion in the dog. Circ. Res. 44, 154-160.

Lohmeier, T.E., Cowley, A.W.,Jr., Trippodo, N.C., Hall, J.E. and Guyton, A.C. (1977). Effects of endogenous angiotensin II on renal sodium excretion and renal hemodynamics. Am. J. Physiol. 233, F388-F395.

Louis, W.J. and Doyle, A.E. (1965). The effects of varying doses of angiotensin on renal function and blood pressure in man and dogs. Clin. Sci. 29, 489-504.

Lowitz, H.D., Stumpe, K.O. and Ochwadt, B. (1969). Micropuncture study of the action of angiotensin II on tubular sodium and water reabsorption in the rat. Nephron 6, 174-187.

Lubash, G.D., Muiesan, G.E., Alicandri, C.L., Garfinkel, D.J., Siekierski, C. and McConnaughey, C.K. (1971). Plasma angiotensin, serum angiotensinase activity and blood pressure response during angiotensin II amide infusions in normal volunteers. Experientia, 27, 68-69.

Ludens, J.H. and Fanestil, D.D. (1976). The mechanism of aldosterone function. Pharmacol. Ther. B. 2, 371-412.

Luft, F.C., Rankin, L.I., Bloch, R., Weyman, A.E., Willis, L.R., Murray, R.H., Grim, C.E. and Weinberger, M.H. (1979). Cardiovascular and humoral responses to extremes of sodium intake in normal black and white men. Circulation 60, 697-706.

Lumbers, E.R. (1971). Activation of renin in amniotic fluid by low pH. Enzymologia 40, 329-336.

Lutz, M.D., Cardinal, J. and Burg, M.B. (1973). Electrical resistance of renal proximal tubule perfused in vitro. Am. J. Physiol. 225, 729-734.

Mahieu, P.R., Foidart, J.B., Du Bois, C.H., Dechenne, C.A. and Deheneffe, J. (1980). Tissue culture of normal rat glomeruli: contractile activity of the cultured mesangial cells. Invest. Cell Pathol. 3, 121-128.

Malvin, R.L. (1971). Possible role of the renin-angiotensin system in the regulation of antidiuretic hormone secretion. Fed. Proc. 30, 1381-1386.

Malvin, R.L. and Vander, A.J. (1967). Effects of angiotensin infusion on renal function in the unanaesthetised rat. Am. J. Physiol. 213, 1205-1208.

Marchand, G.R. (1978). Interstitial pressure during volume expansion at reduced renal artery pressure. Am. J. Physiol. 235, F209-F212.

Marks, E.S., Bing, R.F., Thurston, H. and Swales, J.D. (1980). Vasodepressor property of the converting enzyme inhibitor Captopril (SQ 14,225): the role of factors other than renin-angiotensin blockade in the rat. Clin. Sci. 58, 1-6.

Marshall, G. (1976). Structure-activity relations of antagonists of the renin-angiotensin system. Fed. Proc. 35, 2494-2501.

Martino, J.A. and Earley, L.E. (1967). Demonstration of a role of physical factors as determinants of the natriuretic response to volume expansion. J. Clin. Invest. 46, 1963-1978.

Massey, T.H. and Fessler, D.C. (1976). Substrate binding properties of converting enzyme using a series of p-nitrophenylalanyl derivatives of angiotensin I. Biochemistry 15, 4906-4912.

Matsunaga, M. and Masson, G.M.C. (1970). Hepatic and renal neutral angiotensinases. Experientia 26, 1297-1298.

McAfee, R.D. and Locke, W. (1967). Effects of angiotensin amide on sodium isotope flux and short circuit current of isolated frog skin. Endocrinology 81, 1301-1305.

McCaa, R.E. (1982). Role of the renin-angiotensin-aldosterone system and kallikrein-kinin systems in the control of fluid and elctrolyte metabolism, renal function, and arterial blood pressure. Clin. Exper. Hypertens. A-Theor. 4 (9-10), 1593-1611.

McDonald, J.K., Zeitman, B.B., Callahan, P.X. and Ellis, S. (1974). Angiotensinase activity of dipeptidyl aminopeptidase I (Cathepsin C) of rat liver. J. Biol. Chem. 249, 234-240.

McGiff, J.C. (1980). Interactions of prostaglandins with the kallikrein-kinin and renin-angiotensin systems. Clin. Sci. 59 (Suppl) 105-116.

Menard, J., Breminer, J. and Corvol, P. (1974a). Physicochemical properties of plasma renin substrate in normal, binephrectomized and estrogen treated rats. Am. J. Physiol. 226, 1434-1437.

Menard, J., Corvol, P. and Catt, K.J. (1974b). Steroid contraceptives, renin activity and hypertension. In: Research on Steriods, edited by Breuer, J. Amsterdam, Elsevier/North-Holland biomedical press, pp. 455-467.

Menard, J., Malmejac, A. and Milliez, P. (1970). Influence of diethylstilbestrol on the renin angiotensin system of male rats. Endocrinology 86, 774-780.

Mendelsohn, F.A.O. (1976). A method for measurement of angiotensin II in tissues and its application to rat kidney. Clin. Sci. Mol. Med. 51, 111-125.

Mendelsohn, F.A.O. (1979). Evidence for the local occurrence of angiotensin II in the rat kidney and its modulation by dietary sodium intake and converting enzyme blockade. Clin. Sci. 57, 173-179.

Mendelsohn, F.A.O. (1982). Angiotensin II. Evidence for its role as an intrarenal hormone. Kidney Int. 22 (suppl. 12), S-78-S-81.

Mendelsohn, F.A.O. and Kachel, C.D. (1980). Action of angiotensins I, II and III on aldosterone production by isolated rat adrenal zona glomerulosa cells: Importance of metabolism and conversion of peptides in vitro. Endocrinology 106, 1760-1768.

Michelakis, A.M., Yoshida, H., Menzie, J., Murakami, K. and Inagami, T. (1974). A radioimmunoassay for the direct measurment of renin in mice and it's application to submaxillary gland and kidney studies. Endocrinology 94, 1101-1105.

Misono, K.S., Chang, J.J. and Inagami, T. (1982). Amino acid sequence of mouse submaxillary gland renin. Proc. Natl. Acad. Sci. USA 79, 4858-4862.

Misumi, J., Gonzales, M.F., Ogihara, T., Corvol, P. and Menard, J. (1983). Vascular effects and metabolism of angiotensins and their metabolites in the isolated perfused rat kidney. Clin. Exp. Hypertens. A-Theor. 5 (7-8), 1151-1162.

Moore, A.F., Gurchinoff, S., Brashear, W., Bumpus, F.M., Chang, R. and Khairallah, P.A. (1977). Angiotensinase activity in red blood cell membranes and intact adrenal cells. Res. Commun. Chem. Pathol. Pharmacol. 18, 697-707.

Morgan, T. and Davis, J.M. (1975). Renin secretion at the individual nephron level. Pflugers Arch. 359, 23-31.

Morgan, T. and Gillies, A. (1977). Factors controlling the release of renin: a micropuncture study in the cat. Pflugers Arch. 368, 13-18.

Morris, B.J. and Johnston, C.I. (1976). Renin substrate in granules from rat kidney cortex. Biochem. J. 154, 625-637.

Muller-Suur, R., Gutsche, H-U., Samwer, K.F., Oelkers, W. and Hierholzer, K. (1975). Tubuloglomerular feedback activity in rat kidneys of different renin contents. Pflugers Arch. 359, 33-56.

Munck, O., de Bono, E. and Mills, I.H. (1970). Distribution of blood flow in the renal cortex during saline loading. Clin. Sci. 38, 699-712.

Munday, K.A., Parsons, B.J. and Poat, J.A. (1971). The effect of angiotensin on cation transport by rat kidney cortex slices. J. Physiol. 215, 269-282.

Munday, K.A., Parsons, B.J. and Poat, J.A. (1972). Studies on the mechanisms of action of angiotensin on ion transport by kidney cortex slices. J. Physiol. 224, 195-206.

Murakami, K. and Inagami, T. (1975). Isolation of pure and stable renin from hog kidney. Biochem. Biophys. Res. Commun. 62, 757-763.

Myers, B.D., Deen, W.M. and Brenner, B.M. (1975). Effects of norepinephrine and angiotensin II on the determinants of glomerular ultrafiltration and proximal tubule fluid reabsorption in the rat. Circ. Res. 37, 101-110.

Naftilan, A.J. and Oparil, S. (1978). Inhibition of renin release from rat kidney slices by the angiotensins. Am. J. Phsyiol. 235, F62-F68.

Naftilan, A.J. and Oparil, S. (1981). Effects of sodium intake and Goldblatt hypertension on renin release in rat kidney slices. Am. J. Physiol. 240, F502-F507.

Naftilan, A.J. and Oparil, S. (1982). The role of calcium in the control of renin release. Hypertension 4, 670-675.

Nagatsu, I.T., Yamamoto, T., Glenner, G.G. and Mehl, J.W. (1970). Purification of aminopeptidase A in human serum and degradation of angiotensin II by the purified enzyme. Biochim. Biophys. Acta. 198, 225-270.

Nakajima, T., Oshima, G., Yeh, H.S.J., Igic, R. and Erdos, E.G. (1973). Purification of the angiotensin I converting enzyme of the lung. Biochim. Biophys. Acta. 315, 430-438.

Nasjletti, A. and Masson, G.M.C. (1971). Hepatic origin of renin substrate. Can. J. Physiol. Pharmacol. 49, 931-932.

Nasjletti, A. and Masson, G.M.C. (1972). Studies on angiotensinogen formation in a liver perfusion system. Circ. Res. 30-31 (suppl II), II-187-II-202.

Nasjletti, A. and Masson, G.M.C. (1973). Stimulation of angiotensinogen formation by renin and angiotensin. Proc. Soc. Exp. Biol. Med. 142, 307-310.

Nast, H.P., Walter, U. and Distler, A. (1975). Metabolism of angiotensin II during angiotensin infusion following sodium depletion. Klin. Wochenschr. 53, 623-628.

Navar, L.G. (1984). Renal regulation of body fluid balance. In: Edema, edited by Staub, N.C. and Taylor, A.E. New York, Raven, pp. 319-352.

Navar. L.G., Bell, P.D. and Ploth, D.W. (1981). Role of feedback mechanism in renal autoregulation and sensing step in feedback pathway. Fed. Proc. 40, 93-98.

Navar, L.G., Jirakulsomchok, D., Bell, P.D., Thomas, C.E. and Huang, W-C. (1982). Influence of converting enzyme inhibition on renal hemodynamics and glomerular dynamics in sodium-restricted dogs. Hypertension 4, 58-68.

Navar, L.G., La Grange, R.A., Bell, P.D., Thomas, C.E. and Ploth, D.W. (1979). Glomerular and renal hemodynamics during converting enzyme inhibition (SQ20,881) in the dog. Hypertension 1, 371-377.

Navar, L.G. and Langford, H.G. (1974). Effects of angiotensin on the renal circulation. In: Angiotensin (Handbuch der exp. Pharmakol., vol. 37), edited by Page, I.H. and Bumpus, F.M. Berlin, Springer-Verlag, pp. 455-474.

Navar, L.G., Ploth, D.W. and Bell, P.D. (1980). Distal tubular feedback control of renal hemodynamics and autoregulation. Ann. Rev. Physiol. 42, 557-571.

Neumann, K.H. and Rector, F.C.,Jr. (1976). Mechanism of NaCl and water reabsorption in the proximal convoluted tubule of rat kidney. J. Clin. Invest. 58, 1110-1118.

Ng, K.K.F. and Vane, J.R. (1967). Conversion of angiotensin I to angiotensin II. Nature. 216, 762-766.

Ng, K.K.F. and Vane. J.R. (1968). Fate of angiotensin I in the circulation. Nature 218, 144-150.

Ng, K.K.F. and Vane J.R. (1970). Some properties of angiotensin converting enzyme in the lung. Nature 225, 1142-1144.

Nishimura, K., Hiwada, K., Ueda, E. and Kokubu, T. (1976). Solubilisation of angiotensin I-converting enzyme from rabbit lung using trypsin-treatment. Biochim. Biophys. Acta. 452, 144-150.

Nishimura, K., Yoshida, N., Hiwada, K., Ueda, E. and Kokubu, T. (1978). Properties of three different forms of angiotensin I-converting enzyme from human lung. Biochim. Biophys. Acta. 522, 229-237.

Ogino, K., Matsunaga, M., Saito, N., Kira, J. and Takayasu, M. (1967). Renin and acid adenosine triphosphatase as lysosomal enzymes. Jap. Circ. J. 31, 1-8.

Okahara, T., Abe, T. and Yamamoto, K. (1977). Effects of dibutyryl cyclic AMP and propanolol on renin secretion in dogs. Proc. Soc. Exp. Biol. Med. 159, 213-218.

Ondetti, M.A., Rubin, B. and Cushman, D.W. (1977). Design of specific inhibitors of angiotensin converting enzyme: a new class of orally active antihypertensive agents. Science 196, 441-444.

Ondetti, M.A., Williams, N.J., Sabo, E.F., Pluscec, J., Weaver, E.R. and Kocy, O. (1971). Angiotensin-converting enzyme inhibitors from the venom of BOTHROPS jararaca: Isolation, elucidation of structure, and synthesis. Biochemistry 10, 4033-4039.

Oparil, S. and Bailie, M.D. (1973). Mechanism of renal handling of angiotensin II in the dog. Circ. Res. 33, 500-507.

Oparil, S., Treggear, G.W., Koerner, R., Barnes, B.A. and Haber, E. (1971). Mechanism of pulmonary conversion of angiotensin I to angiotensin II in the dog. Circ. Res. 29, 682-690.

Orekhovich, V.N. (1968). The proteolytic enzymes. Ital. J. Biochem. 17, 241-256.

Osborne, M.J., D'Auriac, A.G., Meyer, P. and Worcel, M. (1970). Mechanism of extraction of angiotensin II in coronary and renal circulations. Life Sci. 9, 859-867.

Osgood, R.W., Lameire, N.H., Sorkin, M.I. and Stein, J.H. (1977). Effects of aortic clamping on proximal reabsorption and sodium excretion in the rat. Am. J. Physiol. 232, F92-F96.

Oshima, G., Gecse, A. and Erdos, E.G. (1974). Angiotensin I converting enzyme of the kidney cortex. Biochim. Biophys. Acta. 350, 26-37.

Oshima, G., Nagasawa, K. and Kato, J. (1976). Renal angiotensin I-converting enzyme as a mixture of Sialo-and Asialo-enzyme, and a rapid purification method. J. Biochem. (Tokyo) 80, 477-483.

Osman, M.Y., Smeby, R.R. and Sen, S. (1979). Separation of dog brain renin-like activity from acid protease activity. Hypertension 1, 53-60.

Oster, P. Hackenthal, E. and Hepp, R. (1973). Radioimmunoassay of angiotensin II in rat plasma. Experientia 29, 353-354.

Ott, C.E., Haas, J.A., Cuche, J-L. and Knox, F.G. (1975). Effect of increased peritubule protein concentration on proximal tubule reabsorption in the presence and absence of extracellular volume expansion. J. Clin. Invest. 55, 612-620.

Ott, C.E., Navar, L.G. and Guyton, A.C. (1971). Pressures in static and dynamic states from capsules implanted in the kidney. Am. J. Physiol. 221, 394-400.

Page, I.H. and Bumpus, F.M. (editors) (1974). Angiotensin (Handbuch der exp. Pharmakol., vol. 37), Berlin, Springer-Verlag.

Page, I.H. and Helmer, O.M. (1940). Angiotonin activator, renin and angiotonin-inhibitor and the mechanism of angiotonin tachyphylaxis in normal, hypertensive and nephrectomised animals. J. Exp. Med. 71, 495-519.

Page, I.H., McSwain, B., Knapp, G.M. and Andrus, W.D. (1941). The origin of renin activator. Am. J. Physiol. 135, 214-222.

Palaic, D. and Farruggia, P. (1980). Separation of angiotensin receptor from cell membrane angiotensinase in rabbit aorta. Clin. Exp. Hypertens. 2, 973-981.

Patchett, A.A., Harris, E., Tristram, E.W., Wyvratt, M.J., Wu, M.T., Taub, D., Peterson, E.R., Ikeler, T.J., Broeke, J.T., Payne, L.G., Ondeyka, D.L., Thorsett, E.D., Greenlee, W.J., Lohr, N.S., Hoffsommer, R.D., Joshua, H., Ruyle, W.V., Rothrock, J.W., Aster, S.D., Maycock, A.L., Robinson, F.M., Hirschmann, R., Sweet, C.S., Ulm, E.H., Gross, D.M., Vassil, T.C. and Stone, C.A. (1980). A new class of angiotensin converting enzyme inhibitors. Nature 288, 280-283.

Peach, M.J. (1971). Adrenal medullary stimulation induced by angiotensin I, angiotensin II and analogues. Circ. Res. 28-29, (suppl II) II-107-II-117.

Peach, M.J. (1981). Molecular actions of angiotensin. Biochem. Pharmacol. 30 (20), 2745-2751.

Peach, M.J. and Chiu, A.T. (1974). Stimulation and inhibition of aldosterone biosynthesis in vitro by angiotensin II and analogs. Circ. Res. 34-35 (suppl. I), I-7-I-13.

Peart, W.S. (1959a). Renin and hypertension. Ergeb. Physiol. 50, 409-432.

Peart, W.S. (1959b). Hypertension and the kidney II. The experimental basis of renal hypertension. Brit. Med. J. II, 1412-1429.

Pelayo, J.C. and Blantz, R.C. (1984). Analysis of renal denervation in the hydropenic rat: interactions with angiotensin II. Am. J. Physiol. 246, F87-F95.

Plentl, A.A., Page, I.H. and Davis, W.W. (1943). The nature of renin activator. J. Biol. Chem. 147, 143-153.

Ploth, D.W. and Roy, R.N. (1982a). Renal and tubuloglomerular feedback effects of (Sar^1,Ala^8) angiotensin in the rat. Am. J. Physiol. 242, F149-F157.

Ploth, D.W. and Roy, R.N. (1982b). Renin-angiotensin influences on tubuloglomerular feedback activity in the rat. Kidney Int. 22 (suppl. 12), S-114-S-121.

Ploth, D.W., Rudolph, J., LaGrange, R. and Navar, L.G. (1979). Tubuloglomerular feedback and single nephron function after converting enzyme (SQ20,881) inhibition in the rat. J. Clin. Invest. 64, 1325-1335.

Poth, M.M., Heath, R.G. and Ward, M. (1975). Angiotensin-converting enzyme in human brain. J. Neurochem. 25, 83-85.

Potkay, S. and Gilmore, J.P. (1973). Autoregulation of glomerular filtration in renin-depleted dogs. Proc. Soc. Exp. Biol. Med. 143, 508-513.

Printz, M.P. and Printz, J.M. (1977). Human angiotensinogen. Purification, partial characterisation and a comparison with animal prohormones. J. Biol. Chem. 252, 1654-1662.

Pullman, T.N., Oparil, S. and Carone, F.A. (1975). Fate of labelled angiotensin II microinfused into individual nephrons in the rat. Am. J. Physiol. 228, 747-751.

Radhi, A.R.A.H., Chapman, B.J. and Munday, K.A. (1982). The effect of prazosin on the actions of low and high doses of angiotensin II amide in the rat. Mol. Physiol. 2, 335-343.

Rector, F.C.,Jr., Giesen, G.V., Kiil, F. and Seldin, D.W. (1964). Influence of expansion of extracellular volume on tubular reabsorption of sodium independent of changes in glomerular filtration rate and aldosterone activity. J. Clin. Invest. 43, 341-348.

Rector, F.C.,Jr., Sellman, J.C., Martinez-Maldonado, M. and Seldin, D.W. (1967). The mechanism of suppression of proximal tubular reabsorption by saline infusion. J. Clin. Invest. 46, 47-56.

Rector, J.B.., Stein, J.H., Bag, W.H., Osgood, R.W. and Ferris, T.F. (1972). Effect of hemorrhage and vasopressor agents on distribution of renal blood flow. Am. J. Physiol. 222, 1125-1131.

Regoli, D. (1972). Studies on the intrarenal action of the renin-angiotensin system. In: Hypertension 1972, edited by Genest, J. and Koiw, E. Berlin, Springer-Verlag, pp.72-81.

Regoli, D., Park, W.K. and Rioux, F. (1974). Pharmacology of angiotensin. Pharmacol. Rev. 26, 69-123.

Regoli, D., Riniker, B. and Brunner, H.R. (1963). The enzymatic degradation of various angiotensin II derivations by serum, plasma and kidney homogenates. Biochem. Pharmacol. 12, 637-646.

Reid, I.A. (1977). Effects of angiotensin II and glucocorticoids on plasma angiotensinogen concentration in the dog. Am. J. Physiol. 232, E234-E236.

Reid, I.A., Brooks, V.L. Rudolph, C.D. and Keil, L.C. (1982). Analysis of the actions of angiotensin on the central nervous system of conscious dogs. Am. J. Physiol. 243, R82-R91.

Reid, I.A., Tu, W.H., Otsuka, K., Assaykeen T.A. and Ganong, W.F. (1973). Studies concerning the regulation and importance of plasma angiotensinogen concentration in the dog. Endocrinology 93, 107-114.

Rentsch, H. P., Ayer, G., Valloton, M., Ziegler, W. and Truniger, B. (1976). Effects of angiotensin II and noradrenaline on intrarenal hemodynamics in the rat. Eur. J. Clin. Invest. 6, 457-464.

Ribeiro, S.A., Corrado, A.P. and Graeff, F.G. (1971). Antinociceptive action of intraventricular bradykinin. Neuropharmacol. 10, 725-731.

Richoux, J.P., Cordonnier, J.L., Bouhnik, J., Clauser, E., Corvol, P., Menard, J. and Grignon, G. (1983). Immunocytochemical localisation of angiotensinogen in rat liver and kidney. Cell Tissue Res. 233, 439-451.

Rightsel, W.A., Okamura, T., Inagami, T.,Pitcock, J.A., Takii, Y., Brooks, B., Brown, P. and Muirhead, E.E. (1982). Juxtaglomerular cells grown as monolayer cell culture contain renin, angiotensin I-converting enzyme, and angiotensins I and II/III. Circ. Res. 50, 822-829.

Robertson, A.L., Smeby, R.R., Bumpus, F.M. and Page, I.H. (1965). Renin production by organ culture of renal cortex. Science 149, 650-651.

Rosivall, L. and Navar, L.G. (1983). Effects on renal hemodynamics of intra-arterial infusions of angiotensins I and II. Am. J. Physiol. 245, F181-F187.

Rosivall, L., Rinder, D.F., Champion, J., Khosla, M.C., Navar, L.G. and Oparil, S. (1983). Intrarenal angiotensin I conversion at normal and reduced renal blood flow in the dog. Am. J. Physiol. 245, F408-F415.

Rosset, E., Scherrer, J.R. and Veyrat, R. (1973). Increased plasma renin substrate concentration in human malignant hypertension. Helv. Med. Acta. 37, 235-250.

Roth, M., Weitzman, A.F. and Piquilloud, Y., (1969). Converting enzyme content of different tissues of the rat. Experientia 25, 1247.

Rouiller, C. and Orci, L. (1971). The structure of the juxtaglomerular complex. In: The Kidney IV, edited by Rouiller, C. and Muller, A.F. New York, Academic Press, pp. 1-80.

Rubin, B., Laffan, R.J., Kotler, D.C., O'Keefe, E., De Maio, D.A. and Goldberg, M.A. (1978). SQ14,225 (D-3-mercapto-2-methylpropanoyl-proline), a novel orally active inhibitor of angiotensin I converting-enzyme. J. Pharmacol. Exp. Ther. 204, 271-280.

Rubin, I. (1972). Purification of hog renin. Properties of purified hog renin. Scand. J. Clin. Lab. Invest. 29, 51-58.

Rumrich, G and Ullrich, K.J. (1968). The minimum requirements for the maintenance of sodium chloride reabsorption in the proximal convolution of the mammalian kidney. J. Physiol. 197, 69p-70p.

Ryan, J.W. (1974). The fate of angiotensin II. In: Angiotensin (Handbuch der exp. Pharmakol., vol.37), edited by Page, I.H. and Bumpus, F.M. Berlin, Springer-Verlag, pp. 81-110.

Ryan, J.W. and Johnson, D.C. (1969). The renin-like enzyme of rabbit uterus. Biochim. Biophys. Acta 191, 386-396.

Ryan, J.W., Niemeyer, R.S., Goodwin, D.W., Smith, U and Stewart, J.M. (1971). Metabolism of (8-L-(^{14}C) phenylalanine)-angiotensin I in the pulmonary circulation. Biochem. J. 125, 921-923.

Ryan, J.W., Ryan, U.S., Schultz, D.R. Witaker, C., Chung, A. and Dorer, F.E. (1975). Subcellular localisation of pulmonary angiotensin converting enzyme (Kininase II). Biochem. J. 146, 497-499.

Ryan, U.S. and Ryan, J.W. (1980). Angiotensin converting enzyme: II. Pulmonary endothelial cells in culture. Environ. Health Persp. 35, 171-180.

Ryan, U.S., Ryan, J.W., Witaker, C. and Chiu, A. (1976). Localisation of angiotensin converting enzyme (Kininase II), II. Immunocytochemistry and immunofluorescence. Tissue and Cell 8, 125-145.

Sancho, J., Re, R., Burton, J., Barger, A.C. and Haber, E. (1976). The role of the renin-angiotensin-aldosterone system in cardiovascular homeostasis in normal human subjects. Circulation 53, 400-405.

Sato, K. (1975). Re-evaluation of micropuncture techniques: Some of the factors which affect the rate of fluid absorption by the proximal tubule. In: Current problems in Clinical Biochemistry. Biochemical Aspects of Renal Function, vol. 4, edited by Angielski, S. and Dubach, U.C. Bern, Stuttgart, Vienna, Hans Huber, pp. 175-187.

Satoh, S., Itsakaichi, O., Ohyama, Y. and Hayashi, M. (1981). A comparison of the vasoconstrictor effects of angiotensin II and angiotensin III in the canine renal vasculature. Arch. Int. Pharmacodyn. Ther. 251, 301-309.

Schafer, J.A. and Andreoli, T.E. (1976). Anion transport process in the mammalian superficial proximal straight tubule. J. CLin. Invest. 58, 500-513.

Schafer, J.A., Patlak, C.S. and Andreoli, T.E. (1975). A component of fluid absorption linked to passive ion flows in the superficial pars recta. J. Gen. Physiol. 66, 445-471.

Schafer, J.A., Patlak, C.S. and Andreoli, T.E. (1977). Fluid absorption and active and passive ion flows in the rabbit superficial pars recta. Am. J. Physiol. 233, F154-F167.

Schafer, J.A., Patlak, C.S., Troutman, S.L. and Andreoli, T.E. (1978b). Volume absorption in the pars recta. II. Hydraulic conductivity coefficient. Am. J. Physiol. 234, F340-F348.

Schafer, J.A., Troutman, S.L. and Andreoli, T.E. (1974). Volume reabsorption, transepithelial potential differences, and ionic permeability properties in mammalian superficial proximal straight tubules, J. Gen. Physiol. 64, 582-607.

Schafer, J.A., Troutman, S.L., Watkins, M.L. and Andreoli, T.E. (1978a). Volume absorption in the pars recta. I. "Simple" active Na^+ transport. Am. J. Physiol. 234, F332-F339.

Schnermann, J. (1981). Localisation, mediation and function of the glomerular vascular response to alterations of distal fluid delivery. Fed. Proc. 40, 109-115.

Schor, N., Ichikawa, I. and Brenner, B.M. (1980). Glomerular adaptations to chronic dietary salt restriction or excess. Am. J. Physiol. 238, F428-F436.

Schor, N., Ichikawa, I. and Brenner, B.M. (1981). Mechanisms of action of various hormones and vasoactive substances on glomerular ultrafiltration in the rat. Kidney Int. 20, 442-451.

Schuster, V.L., Kokko, J.P. and Jacobsen, H.R. (1984). Angiotensin II directly stimulates sodium transport in rabbit proximal convoluted tubules. J. Clin. Invest. 73, 507-515.

Sealey, J.E., Atlas, S.A. and Laragh, J.H. (1980). Prorenin and other large molecular weight forms of renin. Endocr. Rev. 1, 365-391.

Sealey, J.E., Atlas, S.A. and Laragh, J.H. (1983). Prorenin in plasma and kidney. Fed. Proc. 42, 2681-2689.

Sealey, J.E., Moon,C., Laragh, J.H. and Alderman, M. (1976). Plasma prorenin: cryoactivation and relationship to renin substrate in normal subjects. Am. J. Med. 61, 731-738.

Seely, J.F. (1973). Effects of peritubular oncotic pressure on rat proximal tubule electrical resistance. Kidney. Int. 4, 28-35.

Selkurt, E.E., Womach, I. and Dailey, W.N. (1965). Mechanisms of natriuresis and diuresis during elevated renal arterial pressure. Am. J. Physiol. 209, 95-99.

Semple, P.F. (1981). The effects of hemorrhage and sodium depletion on plasma concentrations of angiotensin II and (des-Asp^1) angiotensin II in the rat. Endocrinology 107, 771-773.

Semple, P.F., Boyd, A.S., Dawes, P.M. and Morton, J.J. (1976). Angiotensin II and its' heptapeptide (2-8), hexapeptide (3-8) and pentapeptide metabolities in arterial and venous blood of man. Circ. Res. 39, 671-678.

Semple, P.F. and Morton, J.J. (1976). Angiotensin II and angiotensin III in rat blood. Circ. Res. 38 (suppl. II), 122-126.

Severs, W.B., Daniels, A.E., Smookler, H.H., Kinnard, W.J. and Buckley, J.P. (1966). Interrelationships between angiotensin II and the sympathetic nervous system. J. Pharmacol. Exp. Ther. 153, 530-537.

Shade, R.E., Davis, J.O., Johnson, J.A., Gottshall, R.W. and Spielman, W.S. (1973). Mechanism of action of angiotensin II and antidiuretic hormone on renin secretion. Am. J. Physiol. 224, 926-929.

Silverman, A.J. and Barajas, L. (1974). Effect of reserpine on the juxtaglomerular granular cells and renal nerves. Lab. Invest. 30, 723-731.

Simmons, N.L. (1978). Hormone stimulation of net transepithelial Na transport in cell culture. J. Physiol. 276, 28P-29P.

Simpson, R.U. and Goodfriend, T.L. (1984). Angiotensin and prostaglandin interactions in cultured kidney tubules. J. Lab. Clin. Med. 103, 255-271.

Skeggs, L.T., Dorer, F.E., Kahn, J.R., Lentz, K.E. and Levine, M. (1976). Biochemistry of the renin-angiotensin system and its role in hypertension. Am. J. Med. 60, 737-748.

Skeggs, L.T., Kahn, J.R., Lentz, K.E. and Shumway, N.P. (1957). The preparation, purification and amino acid sequence of a polypeptide renin substrate. J. Exp. Med. 106, 439-453.

Skeggs, L.T., Lentz, K.E., Hochstrasser, J. and Kahn, J.R. (1963). The purification and partial characterisation of several forms of hog renin substrate. J. Exp. Med. 118, 73-98.

Skeggs, L.T., Lentz, K.E., Kahn, J.R. and Hochstasser, H. (1967). Studies on the preparation and properties of renin. Circ. Res. 20-21 (suppl. II), 91-100.

Skeggs, L.T., Lentz, K.E., Kahn, J.R. and Hochstrasser, H. (1968). Kinetics of the reaction of renin with nine synthetic peptide substrates. J. Exp. Med. 128, 13-34.

Skeggs, L.T., Lentz, K.E., Kahn, J.R. and Shumway, N.P. (1958). The synthesis of a tetradecapeptide renin substrate. J. Exp. Med. 108, 283-297.

Skeggs, L.T., Marsh, W.H., Kahn, J.R. and Shumway, N.P. (1954). The existence of two forms of hypertensin. J. Exp. Med. 99, 275-282.

Skinner, S.L., Cran, E.J., Gibson, R., Taylor, R., Walters, W.A.W. and Catt, K.J. (1975). Angiotensins I and II, active and inactive renin, renin substrate, renin activity and angiotensinase in human liquor amnii and plasma. Am. J. Obstet. Gynecol. 121, 626-630.

Skinner, S.L., Lumbers, E.R. and Symonds, E.M. (1972). Analysis of changes in the renin-angiotensin system during pregnancy. Clin. Sci. 42, 479-488.

Smith, U., Ryan, J.W. and Smith, D.S. (1973). Freeze-etch studies of the plasma membrane of pulmonary endothelial cells. J. Cell. Biol. 56, 492-499.

Soffer, R.L., Reza, R. and Caldwell, P.R.B. (1974). Angiotensin-converting enzyme from rabbit pulmonary particles. Proc. Natl. Acad. Sci. USA 71, 1720-1724.

Spinelli, F. and Walther, A. (1979). Modulation by prostaglandins of angiotensin II-induced stimulation of sodium transport in the proximal tubule of the rat. Colloq. INSERM 85, 273-278.

Spitzer, A. and Windhager, E.E. (1970). Effect of peritubular oncotic pressure changes on proximal tubular fluid reabsorption. Am. J. Physiol. 218, 1188-1193.

Sraer, J.D., Sraer, J., Ardaillou, R. and Mimoune, O. (1974). Evidence for renal glomerular receptors for angiotensin II. Kidney Int. 6, 241-246.

Steiner, R.W., Tucker, B.J. and Blantz, R.C. (1979). Glomerular hemodynamics in rats with chronic sodium depletion. J. Clin. Invest. 64, 503-512.

Steven, K. (1974). Effects of peritubular infusion of angiotensin II on rat proximal nephron function. Kidney Int. 6, 73-80.

Steven, K. (1977). Glomerulotubular balance in the rat kidney. Acta Physiol. Scand. Suppl. 447, 7-30.

Steven, K. and Thorpe, D.H. (1977). Angiotensin II: intratubular and renal capillary pressure regulation in the rat kidney. Acta Physiol. Scand. 101, 394-403.

Stevens, R.L., Micalizzi, E.R., Fessler, D.C. and Pals, D.T. (1972). Angiotensin I converting enzyme of calf lung: Method of assay and partial purification. Biochemistry 11, 2999-3007.

Stewart, J.M., Ferreira, S.H. and Greene, L.J. (1971). Bradykinin potentiating peptide PCA-LYS-TRP-ALA-PRO. Biochem. Pharmacol. 20, 1557-1567.

Stowe, N., Schnermann, J. and Hermle, M. (1979). Resetting of nephron filtration rate during pharmacologic interference with the renin-angiotensin and adrenergic systems in rats. Kidney Int. 15, 473-486.

Sweet, C.S., Gross, D.M., Arbegast, P.T., Gaul, S.L., Britt, P.M., Ludden, C.T., Weitz, D. and Stone, C.A. (1981). Antihypertensive activity of N-[(S)-1-(ethoxycarbonyl)-3-phenylpropyl]-L-Ala-L-Pro (MK421), an orally active converting enzyme inhibitor. J. Pharmacol. Exp. Ther. 216, 558-566.

Sybertz, E.J., Baum, T., Ahn, H.S., Nelson, S., Eynon, E., Desiderio, D.M., Puca, K., Becker, F., Sabin, C., Moran, R., Vander Vliet, G., Kastner, B. and Smith, E. (1983). Angiotensin-converting enzyme inhibitory activity of SCH 31846, a new non-sulphydryl inhibitor. J. Cardiovasc. Pharmacol. 5, 643-654.

Szelke, M., Leckie, B.J., Tree, M., Brown, A., Grant, J., Hallett, A., Hughes, M., Jones, D.M. and Lever, A.F. (1982). A potent new renin inhibitor. In vitro and in vivo studies. Hypertension 4, 59-70.

Takada, Y., Hiwada, K., Unno, M. and Kokubu, T. (1982). Immunocytochemical localisation of angiotensin converting enzyme at the ultra-structural level in the human lung and kidney. Biomed. Res. 3, 169-174.

Takii, Y., Takahashi, N., Inagami, T. and Yokosawa, N. (1980). A new form of renin in normal human plasma: "big renin" is a mixture of inactive prorenin and the new high molecular weight renin. Life Sci. 26, 347-353.

Taub, K.J., Caldicott, W.J.H. and Hollenberg, N.K. (1977). Angiotensin antagonists with increased specificity for the renal vasculature. J. Clin. Invest. 59, 528-535.

Taugner, R., Buhrle, C.P., Ganten, D., Hackenthal, E., Hardegg, C., Hardegg, G. and Nobiling, R. (1983). Immunohistochemistry of the renin-angiotensin-system in the kidney. Clin. Exper. Hyper.- Theory and Practice, A5(7&8), 1163-1177.

Taugner, R. and Hackenthal, E. (1981). Angiotensin II in epitheloid (renin containing) cells of rat kidney. Histochemistry 72, 499-509.

Taugner, R., Hackenthal, E., Helmchen, U., Ganten, D., Kugler, P., Marin-Grez, M., Nobiling, R., Unger, T., Lockwald, I. and Keilbach, R. (1982a). The intrarenal renin-angiotensin system: an immunocytochemical study on the localisation of renin, angiotensinogen, converting enzyme and the angiotensins in the kidney of mouse and rat. Klin. Wochenschr. 60, 1218-1222.

Taugner, R., Hackenthal, E., Rix, E., Nobiling, R. and Roulsen, K. (1982b). Immunocytochemistry of the renin-angiotensin system: Renin, angiotensinogen, angiotensin I, angiotensin II, and converting enzyme in the kidneys of mice, rats, and tree shrews. Kidney Int. 22 (suppl. 12), S-33-S-43.

Taugner, R., Marin-Grez, M., Keilbach, R., Hackenthal, E. and Nobiling, R. (1982c). Immunoreactive renin and angiotensin II in the afferent glomerular arterioles of rats with hypertension due to unilateral renal artery constriction. Histochemistry 76, 61-69.

Tewksbury, D.A. and Dart, R.A. (1981). Molecular weight studies on high molecular weight human angiotensinogen. In: Heterogeneity of Renin and Renin Substrate, edited by Sambhi, M. Amsterdam, Elsevier/North-Holland, pp.261-270.

Tewksbury, D.A., Dart, R.A. and Travis, J. (1981). The amino terminal amino acid sequence of human angiotensinogen. Biochem. Biophys. Res. Commun. 99, 1311-1315.

Thurau, K. (1964). Renal hemodynamics. Am. J. Med. 36, 698-719.

Thurau, K. (1974). Intrarenal action of angiotensin. In: Angiotensin (Handbuch der exp. Pharmakol., vol. 37), edited by Page, I.H. and Bumpus, F.M. Berlin, Springer-Verlag, pp. 475-489.

Thurau, K. (1981). Tubulo-glomerular feedback. In: Advances in Physiological Sciences (Kidney and Body Fluids, vol 11), edited by Takacs, L. New York, Pergamon Press Inc., pp. 75-82.

Thurau, K., Granger, A., Mason, J. and Dalheim, H. (1982). Tubular signal for renin activity in the juxtaglomerular apparatus. Kidney Int. 22 (suppl. 12), S-55-S-62.

Thurau, K. and Mason, J. (1974). The intrarenal function of the juxtaglomerular apparatus. In: Kidney and Urinary Tract Physiology, MTP International Review of Science: Physiology, series 6, vol. 1, edited by Thurau, K. London, Butterworth, pp. 357-389.

Thurston, H. and Swales, J.D. (1978). Converting enzyme inhibitor and Saralasin infusion in rats. Circ. Res. 42, 588-592.

Tigerstedt, R. and Bergman, P.G. (1898). Niere und Krieslauf. Skand. Arch, Physiol. 8, 223-271.

Tsai, B.S. and Peach, M.J. (1977). Angiotensin homologs and analogs as inhibitors of rabbit pulmonary angiotensin-converting enzyme. J. Biol. Chem. 252, 4674-4681.

Tsai, B.S., Peach, M.J., Khosla, M.C. and Bumpus, F.M. (1975). Synthesis and evaluation of (des-Asp^1) angiotensin I as a precursor for (des-Asp^1) angiotensin II ("Angiotensin III"). J. Med. Chem. 18, 1180-1183.

Tucker, B.J. and Blantz, R.C. (1983). Mechanism of altered glomerular hemodynamics during chronic sodium depletion. Am. J. Physiol. 244, F11-F18.

Tucker, B.J., Peterson, O.W., Ziegler, M.G. and Blantz, R.C. (1982). Analysis of adrenergic effects of the anesthetics Inactin and α-chloralose. Am. J. Physiol. 243, F253-F259.

Vander, A.J. (1963). Inhibition of distal tubular sodium reabsorption by angiotensin II. Am. J. Physiol. 205, 133-138.

Vander, A.J. (1967). Control of renin release. Physiol. Rev. 47, 359-382.

Vander, A.J. and Geelhoed, G.W. (1965). Inhibition of renin secretion by angiotensin II. Proc. Soc. Exp. Biol. Med. 120, 399-403.

Vandongen, R. and Peart, W.S. (1974). Calcium dependence of the inhibitory effect of angiotensin on renin secretion in the isolated perfused kidney of the rat. Brit. J. Pharmacol. 50, 125-129.

Villarreal, H., De Pascual, J.N., Arcila, H., Lopey-Vinas, C. and Sierra, P. (1964). The action of angiotensin on renal transport of sodium in the dog. Nephron 1, 338-347.

Voigt, J., Wittman-Liebold, R. and Koster, H. (1982). Purification and characterisation of two forms of rat plasma proangiotensin. Eur. J. Biochem. 122, 183-191.

Waldhausl, W.K., Lucas, C.P., Conn, J.W., Lutz, J.H. and Cohen, E.L. (1970). Studies on the partial isolation of human renin. Biochim. Biophys. Acta. 221, 536-548.

Walker, A.M., Bott, P.A., Oliver, J. and MacDowell, M.C. (1941). The collection and analysis of fluid from single nephrons of the mammalian kidney. Am. J. Physiol. 134, 580-595.

Walker, L.A., Buscemi-Bergin, M. and Gellai, M. (1983). Renal hemodynamics in conscious rats: effects of anaesthesia, surgery, and recovery. Am. J. Physiol. 245, F67-F74.

Ward, P.E., Schultz, W., Reynolds, R.C. and Erdos, E.G. (1977). Metabolism of kinins and angiotensins in the isolated glomerulus and brush border of the rat kidney. Lab. Invest. 38, 581-585.

Warnock, D.G. and Burg, M.B. (1977). Urinary acidification: CO_2 transport by the rabbit proximal straight tubule. Am. J. Physiol. 232, F20-F25.

Watson, J.F. (1966). Effects of saline-loading on sodium reabsorption in the dog proximal tubule. Am. J. Physiol. 210, 781-785.

Weidemann, M.J., Hems, D.A. and Krebs, H.A. (1969). Effects of added adenine nucleotides on renal carbohydrate metabolism. Biochem. J. 115, 1-10.

Weigand, K., Weinze, H. and Falge, C. (1977). Synthesis of angiotensinogen by isolated rat liver cells and its regulation in comparison to serum albumin. Biochem. Res. Commun. 75, 102-110.

Weinman, E.J., Kashgarian, M. and Hayslett, J.P. (1971). Role of peritubular protein concentration in sodium reabsorption. Am. J. Physiol. 221, 1521-1528.

Weir, R.J., Brown, J.J., Fraser, R., Lever, A.F., Logan, R.W., McIlswain, G.M., Morton, J.J., Robertson, J.I.S. and Tree, M. (1975). Relationship between plasma renin, renin-substrate, angiotensin II, aldosterone, and electrolytes in normal pregnancy. J. Clin. Endocrinol. Metab. 40, 108-115.

Werle, E., Trautschold, T., Krammer, K. and Schmal, A. (1968). Anreicherung und immunospecifitat des isorenions der glandula submaxillaris der maus. Hoppe-Seylers Z. Physiol. Chem. 349, 1441-1448.

Werle, E., Vogel, R. and Goldbel, L.F. (1957). Uber ein blutdrucksteigerndes prinzip in extracten aus der glandula submaxillaris der weissen maus. Arch. Exp. Pathol. Pharmacol. 230, 235-244.

Wong, P.C. and Zimmerman, B.G. (1980). Mechanism of captopril-induced renal vasodilation in anesthetized dogs after nonhypotensive hemorrhage. Pharmacol. Exper. Ther. 215, 104-109.

Wong, P.C. and Zimmerman, B.G. (1982). Dependence of renal vasodilator effect of Captopril on prevailing plasma renin level in the dog. Clin. Sci. 63, 355-360.

Wright, F.S. and Briggs, J.P. (1979). Feedback control of glomerular blood flow, pressure and filtration rate. Physiol. Rev. 59, 958-1006.

Yang, H.Y.T., Erdos, E.G. and Chiang, T.S. (1968). New enzymatic route for the inactivation of angiotensin. Nature 218, 1224-1225.

Yang, H.Y.T., Erdos, E.G. and Levin, Y. (1970). A dipeptidyl carboxypeptidase that converts angiotensin I and inactivates bradykinin. Biochim. Biophys. Acta. 214, 374-376.

Yang, H.Y.T., Erdos, E.G. and Levin, Y. (1971). Characterisation of a dipeptide hydrolase (kininase II; angiotensin I converting enzyme). J. Pharmacol. Exp. Ther. 117, 291-300.

Yang, H.Y.T. Neff, N.H. (1972). Distribution and properties of angiotensin converting enzyme of rat brain. J. Neurochem. 19, 2443-2450.

Yokosawa, H., Holladay, L.A., Inagami, T., Haas, E. and Murakami, K. (1980). Human renal renin: Complete purification and characterisation. J. Physiol. Chem. 255, 3498-3502.

Young, M.K.,Jr. and Raisz, L.G. (1952). An anthrone method for determination of Inulin in biological fluids. Proc. Soc. Exp. Biol. Med. 80, 771-774.

APPENDIX

[*From the Proceedings of the Physiological Society*, 16–17 *July* 1982
Journal of Physiology. 332. 93–94*P*]

Pressure dependence of angiotensin-induced natriuresis in rats

By P. J. Harris and K. D. Mitchell. *Department of Physiology, University Medical School, Teviot Place, Edinburgh EH8 9AG*

Infusion of pressor doses of angiotensin II (AII) causes a transient decrease in urinary sodium and water excretion followed by marked natriuresis and diuresis (Navar & Langford, 1974). Although the initial phase of this response may be explained by the concomitant decreases in r.b.f. and g.f.r., the mechanism responsible for the natriuresis remains unclear. The possibility of a direct action of angiotensin on proximal tubular sodium reabsorption has been shown previously (Harris & Young, 1977) and is supported by the study of Healy & Elliott (1971) who showed that angiotensin-induced natriuresis in rabbits occurs even when the increase in blood pressure is prevented by haemorrhage.

Male Wistar rats (250–300 g) were anaesthetized with Inactin (120 mg/kg) and the left kidney prepared for micropuncture (György, Lingard & Young, 1974). A jugular vein was cannulated for infusion of 0·9 % saline (0·075 ml/min) and for administration of AII (Hypertensin, Ciba). A cannula was placed in the exposed ureter and urine collected during 20-min periods. The sodium concentration of the urine was measured by flame photometry. The left femoral or carotid artery was cannulated to enable measurement of mean arterial blood pressure (M.A.B.P.). In a control group of animals ($n = 6$) saline was infused for an initial period and then AII added to the infusate and administered at the rate of 100 ng/min for 60 min. A second period of saline infusion followed. Proximal tubular fluid reabsorption ($J_{v(a)}$) was measured at intervals during each period using the shrinking droplet technique (György, 1971). In a second group of animals ($n = 7$) a snare was placed around the aorta between the origins of the renal arteries. The snare was tightened during the infusion of AII to prevent the rise in systemic blood pressure from raising the perfusion pressure of the left kidney. All data were analysed by Student's paired t-test. In the control group M.A.B.P. increased from 112·4 ± 5·4 mmHg during the control period to 136·0 ± 5·8 during AII infusion ($P < 0·005$). This was associated with an initial fall in urinary sodium excretion ($U_{Na} \cdot \dot{V}$ μmol.min) followed by a significant ($P < 0·05$) increase to almost three times the control value. In the second group femoral arterial blood pressure was not altered during AII infusion, while $U_{Na} \cdot \dot{V}$ was significantly reduced ($P < 0·05$). No significant change in $J_{v(a)}$ was observed during AII infusion in the first group but a significant reduction ($P < 0·05$) occurred in the second group.

These data do not support the proposal that the mechanism of angiotensin-induced natriuresis involves a direct action of angiotensin on sodium reabsorption at least in the proximal tubule. In contrast to previous studies in the rabbit the natriuretic response in the rat appears to be dependent upon the increase in M.A.B.P.

This work was supported by the M.R.C.

[P.T.O.

more detailed investigation of possible medullary action of angiotensin II.

Furosemide Releases Renin in the Rat Kidney when Prostacyclin Synthesis is Suppressed

Robert Vanaongen, Denis P. Manonev, Anne E. Baraen, Anne M. Tunnev
University Department of Medicine, Royal Perth Hospital, Perth, Western Australia

The effect of inhibiting prostaglandin synthesis by indomethacin on basal and furosemide-stimulated renin secretion was examined in the isolated perfused rat kidney. 6-keto-$PGF_{1\alpha}$, the stable PGI_2 derivative, was measured by radioimmunoassay in urine collected from the kidney before and during perfusion. During perfusion, levels of 6-keto-$PGF_{1\alpha}$ were similar when measured directly or after organic extraction and chromatographic separation. Treatment of rats with indomethacin (3.0 mg/kg) reduced 6-keto-$PGF_{1\alpha}$ excretion in the isolated kidney from 121.3 ± 39.1 to 15.1 ± 6.6 pg/min ($n = 9$; $p < 0.02$) but had no effect on basal renin secretion. Renal perfusion pressure, perfusate flow rate and vascular resistance were similar in indomethacin-treated and control rats. Mean urine flow rate was lower after indomethacin treatment. Infusion of furosemide (250 µg/min) did not alter 6 keto-$PGF_{1\alpha}$ excretion in control or indomethacin-treated ($p > 0.05$) rats. Although renin secretion was markedly increased during furosemide infusion, there was no significant difference between control (1.806 ± 384 ngAI/min) and indomethacin-treated (2.310 ± 554 ngAI/min) rats ($p > 0.05$). Propranolol, infused at a dose (8 µg/min), which suppressed a comparable rise in renin secretion after isoproterenol stimulation, did not impair the response to furosemide in indomethacin-treated rats. These results demonstrate that furosemide-stimulated renin secretion in the rat kidney does not require intact renal PGI_2 synthesis and is independent of beta-adrenergic mechanisms.

The Role of Angiotensin in the Regulation of Proximal Tubular Transport

P. J. Harris, K. D. Mitchell
Department of Physiology, University Medical School, Teviot Place, Edinburgh, UK

Previous studies have shown that the stimulatory action of angiotensin II (AII) on proximal reabsorption in the anaesthetised rat appears to be maximal at physiological concentrations. Higher doses of AII inhibit reabsorption. We have examined the effects of i.v. administration of subpressor doses of AII on proximal fluid reabsorption (Jv) and whole kidney function in Inactin-aesthetised rats infused with saline at a moderate rate ($75\ \mu l \cdot min^{-1}$). At 0.1, 1.5, and 10 ng · min^{-1} AII had no significant effects on urine flow or sodium excretion although with all doses GFR decreased. Jv ($\times 10^{-4}\ mm^3 \cdot mm^{-2} \cdot sec^{-1}$ was measured by the shrinking droplet micropuncture technique. In 6 rats Jv decreased from 2.64 ± 0.32 during saline infusion to 2.22 ± 0.26 (NS) during infusion of 0.1 ng · min^{-1} AII. In a separate group of 7 rats Jv was reduced from 2.91 ± 0.23 during saline infusion to 2.24 ± 0.18 (NS) with 1 ng · min^{-1} AII. $2.20_{\pm}0.24$(NS) with 5 ng · min^{-1} and 1.99 ± 0.10 ($p < 0.05$) with 10 ng · min AII. Thus in the anaesthetised rat, small (subpressor) increases in plasma AII concentration have a dose-dependent inhibitory action on proximal tubular reabsorption.

Role of Angiotensin II in Mediating the Actions of the Renal Nerves in the Rat Kidney

E. J. Johns, R. K. Handa
Department of Physiology, The Medical School, Birmingham, UK

The importance of angiotensin II in modulating the actions of the renal nerves in the regulation of renal haemodynamics and tubular sodium reabsorption was examined. The renal nerves were stimulated to cause a modest or no fall in renal blood flow (RBF) in the absence or presence of captopril. Rats were prepared for study as previously described *[Ball and Johns, J. Endocr. 90:* 60 (1982)]. Renal nerve stimulation caused a 15% fall in RBF, no change in glomerular filtration rate (GFR) but reduced absolute and fractional sodium excretions by 37 and 34%, respectively. During infusion of captopril (0.38 m*M*/kg/h) stimulation of the nerves caused a reduction of 16% in both RBF and GFR, a 31% fall in absolute and only an 18% fall in fractional sodium excretion. Nerve stimulation which did not change RBF or GFR reduced absolute and fractional sodium excretions by 26 and 23%, respectively, and captopril abolished these responses. These data support a role for locally generated angiotensin II in the regulation of GFR by acting preferentially at the efferent arteriole